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Cultured mammalian cells, COS-7, NIH3T3, HeLa 1-6, 293, CHO, MDA, MBA1, PM2 and insect cells

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</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction to Protein Extraction</strong></td>
</tr>
<tr>
<td><strong>Cell Lysis Methods</strong></td>
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<td><strong>Introduction to Poppers’ Cell Lysis Solutions</strong></td>
</tr>
<tr>
<td><strong>B-PER® Bacterial Protein Extraction Reagents</strong></td>
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<tr>
<td><strong>Y-PER® Yeast Protein Extraction Reagents</strong></td>
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<td><strong>Mitochondria Isolation Kits</strong></td>
</tr>
<tr>
<td><strong>NE-PER® Nuclear and Cytoplasmic Extraction Kit</strong></td>
</tr>
<tr>
<td><strong>Fusion Protein Purification</strong></td>
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<td><strong>Y-PER® Yeast Protein Extraction Reagents</strong></td>
</tr>
<tr>
<td><strong>T-PER® Tissue Protein Extraction Reagents</strong></td>
</tr>
<tr>
<td><strong>Lyse-N-Go™ PCR Reagent</strong></td>
</tr>
<tr>
<td><strong>Protein Desalting</strong></td>
</tr>
</tbody>
</table>

Poppers’ Cell Lysis Reagents Selection Guide

Introduction to Protein Extraction

Cell Lysis Methods

Introduction to Poppers’ Cell Lysis Solutions

B-PER® Bacterial Protein Extraction Reagents

Y-PER® Yeast Protein Extraction Reagents

Mitochondria Isolation Kits

NE-PER® Nuclear and Cytoplasmic Extraction Kit

Poppers’ Cell Lysis Reagents Selection Guide

Table 1: Poppers’ Cell Lysis Reagents selection guide.

<table>
<thead>
<tr>
<th>Poppers</th>
<th>Product No.</th>
<th>Organisms/Samples</th>
<th>Dialysis Compatibility</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-PER®</td>
<td>6-7</td>
<td>E. coli strains: BL21 (DE3)</td>
<td>Yes</td>
<td>Reporter assays, IP; Western blot, GST- and histidine-tag purification</td>
</tr>
<tr>
<td>T-PER®</td>
<td>9</td>
<td>E. coli strain: BL21 (DE3)</td>
<td>Yes</td>
<td>Reporter assays, IP; Western blot, GST- and histidine-tag purification</td>
</tr>
<tr>
<td>Y-PER®</td>
<td>8</td>
<td>S. cerevisiae, Schizosaccharomyces pombe, C. albicans, B. subtilis, E. coli, P. pastoris, Strep. avidinii, Acetobacter sp.</td>
<td>Yes</td>
<td>IP; Western blot, β-gal enzyme assays, IEF after dialysis, GST- and histidine-tag purification</td>
</tr>
<tr>
<td>Mem-PER®</td>
<td>9</td>
<td>Human, mouse, rat, bovine, rabbit, chicken, yeast, E. coli, S. cerevisiae</td>
<td>Yes</td>
<td>Western blot, GST- and histidine-tag purification, the addition of up to 2 M NaCl may result in increased efficiency of lysis and protein yield</td>
</tr>
<tr>
<td>NE-PER®</td>
<td>24-25</td>
<td>Rat brain, rat heart, rat liver, rat kidneys, rat spleen, rat thymus, rat pancreas, rat lung, rat muscle, rat testes, mouse brain, mouse heart, mouse liver, mouse kidney, mouse spleen, mouse thymus, mouse pancreas, mouse lung, mouse muscle, mouse testes, mouse pituitary, mouse adrenal, mouse gonads, mouse intestine, rat thymus, rat pancreas, rat lung, rat muscle, rat testes, mouse brain, mouse heart, mouse liver, mouse kidney, mouse spleen, mouse thymus, mouse pancreas, mouse lung, mouse muscle, mouse testes, mouse pituitary, mouse adrenal, mouse gonads, mouse intestine</td>
<td>Yes</td>
<td>Luciferase, β-gal (low signal), CAT, kinase assays, ELISAs, immobilized glutathione, Western blot</td>
</tr>
<tr>
<td>Pro-Matrix®</td>
<td>33</td>
<td>Cultured cells: epithelial (HeLa), fibroblasts (NIH3T3), yeast (S. cerevisiae)</td>
<td>Yes</td>
<td>Western blot and 2-D Western blot, reporter assays, ELISAs, immobilized glutathione, Western blot</td>
</tr>
<tr>
<td>Mitochondria Isolation Kit</td>
<td>98874</td>
<td>Mammalian cells</td>
<td>NA</td>
<td>Western blot, 2-D Western blot, reporter assays, applications include apoptosis, signal transduction and metabolic studies</td>
</tr>
<tr>
<td>Mitochondria Isolation Kit</td>
<td>98861</td>
<td>Heart, liver, kidney and brain</td>
<td>NA</td>
<td>Western blot, 2-D Western blot, reporter assays, applications include apoptosis, signal transduction and metabolic studies</td>
</tr>
</tbody>
</table>

Notes:

1. The detergent can be removed by dialysis.
2. Immuno precipitation.
3. HPF: Permits the addition of detergent.
4. Samples prepared in Mem-PER Reagent can be dialyzed if the buffer contains detergent (e.g., CHAPS).
5. Spin & Load™: Membrane Protein Extraction Kit (Product # 99884) and Mem-PER® Reagents.
6. E. coli strains: BL21 (DE3) | Yes | Reporter assays, IP; Western blot, GST- and histidine-tag purification |

Note: Poppers’ Technology is protected by U.S. patent # 6,174,704. U.S. patent pending on Mitochondria Isolation Kit Technology.

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
Introduction to Protein Extraction

Protein purification encompasses total protein extraction from a sample (lysis), specific enrichment and/or isolation of a particular protein of interest (affinity purification), and removal of interfering or contaminating substances (sample preparation or clean-up).

Cell lysis is the first step in cell fractionation and protein purification and, as such, opens the door to a myriad of biological studies. Many techniques are available for the disruption of cells, including physical and detergent-based methods. Historically, physical lysis has been the method of choice for cell disruption; however, physical methods often require expensive, cumbersome equipment and involve protocols that can be difficult to repeat due to variability in the apparatus (such as loose-fitting compared with tight-fitting homogenization pestles). In recent years, detergent-based lysis has become very popular due to ease of use, low cost and efficient protocols. Pierce offers several detergent-based Poppers™ Reagents for the preparation of whole and fractionated cell lysates that are faster and more convenient than traditional lysis methods.

All cells have a plasma membrane, a protein-lipid bilayer that forms a barrier separating cell contents from the extracellular environment. Lipids constituting the plasma membrane are amphiphilic, having hydrophilic and hydrophobic moieties that associate spontaneously to form a closed bimolecular sheet (Figure 1). Membrane proteins are embedded in the lipid bilayer, held in place by one or more domains spanning the hydrophobic core. In addition, peripheral proteins bind the inner or outer surface of the bilayer through interactions with integral membrane proteins or with polar lipid head groups. The nature of the lipid and protein content varies with cell type.

In animal cells, the plasma membrane is the only barrier separating cell contents from the environment. In plants and bacteria, the plasma membrane is also surrounded by a rigid cell wall. Bacterial cell walls are composed of peptidoglycan. Yeast cell walls are composed of two layers of β-glucan, the inner layer being insoluble to alkaline conditions. Both of these are surrounded by an outer glycoprotein layer rich in the carbohydrate mannann. Plant cell walls consist of multiple layers of cellulose. These types of extracellular barrier confer shape and rigidity to the cells. Plant cell walls are particularly strong, making them very difficult to disrupt mechanically or chemically. Until recently, efficient lysis of yeast cells required mechanical disruption using glass beads. Bacterial cell walls are the easiest to break compared to these other cell types. The lack of an extracellular wall in animal cells makes them relatively easy to lyse. Clearly, the technique chosen for the disruption of cells, whether physical or detergent-based, must take into consideration the origin of the cells or tissues being examined and the inherent ease or difficulty in disrupting their outer layer(s). In addition, the method must be compatible with the amount of material to be processed and the intended downstream applications. This handbook discusses both non-detergent and detergent-based lysis techniques and then introduces Poppers™ Cell Lysis Solutions.

Table 2: Techniques used for the physical disruption of cells.

<table>
<thead>
<tr>
<th>Lysis Method</th>
<th>Apparatus</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>Waring® Blender, Polytron® Mixer</td>
<td>Rotating blades grind and disperse cells and tissues</td>
</tr>
<tr>
<td>Liquid Homogenization</td>
<td>Dounce Homogenizer, Potter-Elvehjem Homogenizer, French Press</td>
<td>Cell or tissue suspensions are sheared by forcing them through a narrow space</td>
</tr>
<tr>
<td>Freezing/Freeze</td>
<td>Freezer or dry ice/ethanol</td>
<td>Repeated cycles of freezing and thawing disrupt cells through ice crystal formation</td>
</tr>
<tr>
<td>Manual Grinding</td>
<td>Mortar and pestle</td>
<td>Grinding plant tissues, frozen in liquid nitrogen</td>
</tr>
</tbody>
</table>

Sonication

Sonication is the third class of physical disruption commonly used to break open cells. The method uses pulsed, high-frequency sound waves to agitate and lyse cells, bacteria, spores, and finely diced tissue. The sound waves are delivered using an apparatus with a vibrating probe that is immersed in the liquid cell suspension. Mechanical energy from the probe initiates the formation of microscopic vapor bubbles that form momentarily and implode, causing shock waves to radiate through a sample. To prevent excessive heating, ultrasonic treatment is applied in multiple short bursts to a sample immersed in an ice bath. Sonication is best suited for samples < 100 ml.

Freeze/Thaw

Freeze/thaw method is commonly used to lyse bacterial and mammalian cells. The technique involves freezing a cell suspension in a dry ice/ethanol bath or freezer and then thawing the material at room temperature or 37°C. This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing. Multiple cycles are necessary for efficient lysis, and the process can be quite lengthy. However, freeze/thaw has been shown to effectively release recombinant proteins located in the cytoplasm of bacteria and is recommended for the lysis of mammalian cells in some protocols.
Mortar and Pestle

Manual grinding is the most common method used to disrupt plant cells. Tissue is frozen in liquid nitrogen and then crushed using a mortar and pestle. Because of the tensile strength of the cellulose and other polysaccharides constituting the cell wall, this method is the fastest and most efficient way to access plant proteins and DNA.

Additives/Facilitators

Cells can be treated with various agents to aid the disruption process. Lysis can be promoted by suspending cells in a hypotonic buffer, which causes them to swell and burst more readily by physical shearing. Lysozyme (200 µg/ml) (Product # 89833 and 99834; page 6) can be used to digest the polysaccharide component of yeast and bacterial cell walls. Alternatively, processing can be expedited by treating cells with glass beads in order to facilitate the crushing of cell walls. This treatment is commonly used with yeast cells. Viscosity of a sample typically increases during lysis due to the release of nucleic acid material. DNase (Product # 89835; page 6) can be added to samples (25-50 µg/ml) along with RNase (50 µg/ml) to reduce this problem. DNase (Product # 89833 and 89834; page 6) can be used to digest the cellulose and other polysaccharides constituting the cell wall, this method is the fastest and most efficient way to access cell and its contents using Y-PER® Yeast Protein Extraction Reagent (Product # 78990) (Figure 3). The method does not require enzymes or glass beads to aid cell lysis and results in the release of functionally active solubilized proteins. The reagent can also be used effectively with gram-positive and gram-negative bacteria. Y-PER® Plus DIALYZABLE Yeast Protein Extraction Reagent (Product # 78999) is an alternative to the original Y-PER® Reagent. It is phosphate-free and has a much lower ionic strength than the original Y-PER® Reagent.

The whole cell lysates prepared with all of the above-mentioned Poppers™ Reagents except Y-PER® Reagent are compatible with BCA® and Coomassie Protein Assays.

**Introduction to Poppers™ Cell Lysis Solutions**

To eliminate the need for hit-or-miss homemade recipes, Pierce offers Poppers™ Cell Lysis Reagents for lysing various sample types (Table 1, page 1). Mammalian cells are one of the easiest cell types to lyse. The M-PER® Mammalian Protein Extraction Reagent (Product # 78501) uses a non-denaturing detergent to prepare total cell lysate that is compatible with many downstream assays including immunoenzymes, enzyme assays and a variety of common reporter assays. The major advantage to the M-PER® Reagent is that lysis can be performed directly on the plate and is completed in only 5 minutes. Furthermore, significantly more protein can be obtained with this method compared with freeze/thaw and sonication (Figure 2).

Detergents break the lipid barrier surrounding cells by solubilizing proteins and disrupting lipid, protein and protein:lipid interactions. Detergents, like lipids, self-associate and bind to hydrophobic surfaces. They are composed of a polar hydrophilic head group and a nonpolar hydrophobic tail and are categorized by the nature of the head group as either ionic (cationic or anionic), nonionic or zwitterionic. Their behavior depends on the properties of the head group and tail.

Unfortunately, there is no standard protocol available for selecting a detergent to use for membrane lysis. The ideal detergent will depend on the intended application. In general, nonionic and zwitterionic detergents are milder and less denaturing than ionic detergents and are used to solubilize membrane proteins when it is critical to maintain protein function and/or retain native protein:protein interactions for functional assays or immunoenzymes. CHAPS, a zwitterionic detergent, and the Triton X® Brand series of nonionic detergents are commonly used for these purposes. In contrast, ionic detergents are strong solubilizing agents and tend to denature proteins, thereby destroying protein activity and function. Studies assessing protein levels strictly through gel electrophoresis are commonly used as a method to evaluate protein:protein interactions for functional assays. However, this method offers limited information about the specificity of protein-protein interactions and/or retain native protein:protein interactions for functional assays.

The choice of detergent for cell lysis also depends on sample type. Animal cells, bacteria and yeast all have differing requirements for optimal lysis due to the presence or absence of a cell wall. Because of the dense and complex nature of animal tissues, they require both detergent and mechanical lysis. In addition to the choice of detergent, other important considerations for optimal cell lysis include the buffer, pH, salt concentration and temperature. Consideration should be given to the compatibility of the chosen detergent with downstream applications. If the detergent used for lysis must be removed, then a dialyzable detergent should be selected.

**References**


**Figure 2**. Comparison of M-PER® Reagent with freeze/thaw cycles, sonication and Brand P lysis buffer. COS-7 cells grown in 100 mm plates at full confluency were washed once with 10 ml of PBS, scraped with 1 ml of PBS and centrifuged at 5,000 rpm for 5 minutes to collect the cells. The cell pellets were resuspended in 0.5 ml of respective extraction reagents and subjected to total protein extraction. For freeze/thaw cycles, the cell suspension in PBS was frozen in a dry ice and isopropyl alcohol bath for 10 minutes and thawed in a 37°C water bath. The freeze/thaw cycle was repeated three times. For sonication, the cell suspension was sonicated for 2 minutes with a 50% pulse using a Branson Sonifier® 450 Sonicator. For extraction with M-PER® Reagent and Brand P lysis buffer, the cell suspensions were shaken for 5 minutes. The cell debris was removed by centrifugation at 13,000 rpm for 5 minutes and the supernatants were assayed for protein concentration by the BCA® Method.

**Figure 3**. Y-PER® Reagent significantly disrupts yeast cell wall and plasma membrane. Cells of Saccharomyces cerevisiae strain DY150 after lysis with Y-PER® Yeast Protein Extraction Reagent. Arrows indicate disruption of cell wall, resulting in cell lysis.
B-PER® Bacterial Protein Extraction Reagent

Yields obtained with B-PER® Reagent greatly exceed those obtained using standard saccharide methods.

Highlights:
- Recovers both soluble and insoluble recombinant protein from bacterial lysates—puriﬁes inclusion bodies to near-homogeneous levels
- One easy-to-use reagent—B-PER® Reagent provides one-step E. coli cell lysis by a mild, nonionic detergent in 20 mM Tris-Cl buffer (pH 7.5)
- Fast and simple—just add B-PER® Reagent to a bacterial pellet and shake for 10 minutes. Recover soluble proteins by pelleting cell debris. Purify inclusion body* from the pellet using an optimized procedure
- Flexible—B-PER® Reagent is suitable for any scale protein extraction
- Avoids contamination—B-PER® Reagent is free of enzymatic components, thus avoiding contamination of your recombinant protein. If necessary, the nonionic detergent can be removed by dialysis
- Compatible with GST, 6xHis and other afﬁnity puriﬁcations

Reagents effectively extract and remove soluble proteins from the insoluble fraction. They are especially suitable for the commonly used, proteinase-deﬁcient bacterial expression host BL21 strains. If the lysis is not efﬁcient for a particular strain, the expression host BL21 strains: 

If the lysis is not efﬁcient for a particular strain, the expression host BL21 strains: 

B-PER® Reagent has been tested for the extraction of recombinant proteins from insect cells infected by baculovirus. The amount of the reagent required depends on the conﬁdence of the infected cells.

Table 3. General considerations for B-PER® Reagent

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>78248</td>
<td>B-PER® Bacterial Protein Extraction Reagent</td>
<td>500 ml</td>
</tr>
<tr>
<td>78243</td>
<td>B-PER® Bacterial Protein Extraction Reagent</td>
<td>150 ml</td>
</tr>
<tr>
<td>39633</td>
<td>Lysozyme</td>
<td>5 g</td>
</tr>
<tr>
<td>39634</td>
<td>Lysozyme</td>
<td>25 g</td>
</tr>
<tr>
<td>39635</td>
<td>DNase I</td>
<td>500 units</td>
</tr>
</tbody>
</table>

* Does not solubilize inclusion bodies.

Optional/supplemental materials:
- Protease inhibitors, salts, chelating agents, reducing agents, etc. may be added directly to the reagent for speciﬁc applications.

Figure 4. Comparison of B-PER® Reagent with sonication. E. coli expressing GFP was extracted ﬁve times with B-PER® Reagent or PBS/sonication. Each extraction was analyzed by SDS-PAGE.

Rounds of Extraction

<table>
<thead>
<tr>
<th>Rounds of Extraction</th>
<th>B-PER® Reagent</th>
<th>PBS/sonication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>300</td>
</tr>
</tbody>
</table>

Figure 5. Comparison of B-PER® Reagent for measurement of green ﬂuorescent protein (GFP). E. coli expressing GFP was extracted ﬁve times with B-PER® Reagent or PBS/sonication. Each extraction was analyzed by GFP activity assay.

<table>
<thead>
<tr>
<th>Rounds of Extraction</th>
<th>B-PER® Reagent</th>
<th>PBS/sonication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

** GFP = Green Fluorescent Protein

Figure 6. Comparison of B-PER® Reagent (in Phosphate Buffer) with original B-PER® Reagent. Approximately 1 g of bacterial pellets was extracted ﬁve times with 10 ml of either B-PER® II Bacterial Protein Extraction Reagent (in Phosphate Buffer) or original B-PER® Reagent. B-PER® Reagent (in Phosphate Buffer) showed a pattern similar to that of the original B-PER® Reagent, i.e., most of the proteins were extracted in the first round. The protein concentration is measured by (A) BCA® Assay and the green ﬂuorescent protein (GFP) activity is determined by a (B) luminescence spectrometer (Perkin-Emer L550).

Ordering Information

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>78296</td>
<td>B-PER® Reagent (in Phosphate Buffer)</td>
<td>500 ml</td>
</tr>
</tbody>
</table>

B-PER® II Bacterial Protein Extraction Reagent

Developed to provide more efﬁcient recombinant protein extraction with smaller volumes than that of the original B-PER® Reagent.

Highlights:
- Can be used for soluble protein extraction and inclusion body purification
- Protein remains concentrated
- Uses Trit buffer system
- No need for expensive equipment—complete lysis achieved with B-PER® II Bacterial Protein Extraction Reagent

Ordering Information

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>78250</td>
<td>B-PER® II Bacterial Protein Extraction Reagent</td>
<td>500 ml</td>
</tr>
</tbody>
</table>

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
**M-PER® Mammalian Protein Extraction Reagent**

**Designed to provide highly efficient total protein extraction from cultured mammalian cells.**

**Highlights:**
- Mild detergent lysis, yielding extracts that are immediately compatible with Coomassie (Bradford) and BCA® Protein Assays or SDS-PAGE®
- Extracts soluble proteins in nondenatured state, enabling direct use in immunoprecipitation and other affinity purification procedures
- Amine-free and fully dialyzable formulation ensures compatibility with subsequent assay systems
- Lyse adherent cells directly in plate or after scraping and washing in suspension
- Maintain luciferase, β-galactosidase, CAT and other reporter gene activities as well or better than competitor products and freeze/thaw methods

To achieve complete cell lysis a unique detergent was chosen that dissolves cell membranes at low concentrations, does not denature protein and is compatible with downstream assays. M-PER® Reagent extracts 25% and 20% more protein than freeze/thaw cycles and sonication, respectively (Figure 2, page 5).

**M-PER® Reagent is suitable for cell lysis on all sizes of culture plates**

Lysis with M-PER® Reagent is so efficient that adherent cells do not need to be scraped from the culture dish, especially important when the cells are grown in small-well plates, such as 96- or 24-well plates.

Total protein was recovered efficiently without scraping the cells by simply adding an appropriate amount of M-PER® Reagent and shaking for 5 minutes as compared with the Brand P Providence buffer in 100 mm, 60 mm, 6-well, 24-well and 96-well plates. (Data available on the Pierce web site.) This feature also provides the feasibility for high-throughput cell lysis and subsequent screening assays.

**M-PER® Reagent is compatible with reporter assays, kinase assays, immunosassays and protein assays**

M-PER® Reagent is compatible with (A) luciferase, (B) β-galactosidase and (C) CAT assays. Three popular gene regulation reporter assays (Figure 8). Compared to lysing with Brand P lysis buffer followed by one freeze/thaw cycle (as suggested by the manufacturer) or the standard freeze/thaw method, M-PER® Reagent yielded more or equivalent enzyme activities.

**Use Y-PER® Plus Reagent to extract functional soluble proteins and proteins from S. cerevisiae and P. pastoris (yeast), E. coli (Gram-positive bacteria), and E. coli (Gram-negative bacteria).**

Protein extraction is achieved by a 20-minute incubation in the reagent at room temperature.

**Ordering Information**

**Product #** | **Description** | **Pkg. Size**
---|---|---
75998 | M-PER® Plus Dialyze Yeast Protein Extraction Reagent | 25 ml
75999 | M-PER® Plus Dialyze Yeast Protein Extraction Reagent | 500 ml

---

**References**


Y-PER® Yeast Protein Extraction Reagent

Easy-to-use solution gently disrupts the tough yeast cell wall in less than 20 minutes at room temperature.

Highlights:

- Extracts more than twice as much protein as glass bead methods (Figure 9)
- Eliminates the physical problems associated with traditional glass bead lysis (e.g., clinging static-charged beads, protein/bead clumps and runaway beads)
- Works with Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris and Bacillus subtilis
- Effective for many different organisms, including gram-positive and gram-negative bacteria (Figure 10); suitable for use in a diverse range of situations

Traditionally, protein extraction from yeast required physical disruption to break through the thick proteinaceous cell envelope; less disruptive lysis methods were possible only with other organisms like E. coli. Y-PER® Yeast Protein Extraction Reagent was the first commercially available yeast lysis reagent to use a mild detergent lysis procedure to efficiently release functionally active solubilized proteins. Several uses for the Y-PER® Reagent have been optimized that encompass a broad array of applications ranging from fusion-tagged protein purification to microplate-compatible enzyme assays, and genomic and plasmid DNA extraction from yeast. Y-PER® Reagent has even been used to isolate yeast killer virus double-stranded RNA from killer strains of S. cerevisiae.

Protocol

The standard protocol for protein extraction is easy:

1. Add an appropriate volume of Y-PER® Reagent to pelleted yeast cells.
2. Incubate at room temperature for approximately 20 minutes.
3. Spin down the debris.

In all three organisms tested, Y-PER® Reagent extracts contain more usable protein than traditional glass bead lysis.

Figure 9. Y-PER® Reagent extraction yields greater amounts of usable protein.

In all three organisms tested, Y-PER® Reagent extracts contain more usable protein than traditional glass bead lysis.

Y-PER® Yeast Protein Extraction Reagent

Optimized high-capacity purifications.

Highlights of both kits:

- Greater convenience — no sonication required; complete cell lysis achieved with B-PER® Reagent
- Ready-to-use components

Highlights of the B-PER® 6xHis Fusion Protein Column Purification Kit:

- Optimized system provides the best purity in the least amount of time (works in 2.5-3 hours)
- High capacity — kit makes it possible to purify more than 10 mg of over-expressed protein per column

Figure 10. Y-PER® Yeast Protein Extraction Reagent. Y-PER® Yeast protein extraction of protein from two different strains each of S. cerevisiae, S. pombe and B. subtilis.

Highlights of the B-PER® 6xHis Fusion Protein Spin Purification Kit:

- Works in less than 30 minutes
- Includes an optimized protocol that makes it possible to achieve yields approximately 1 mg of pure 6xHis-tagged fusion protein
- Includes Handee™ Spin Columns and Collection Tubes

The B-PER® 6xHis Column and Spin Purification Kits are designed for rapid and efficient purification of 6xHistidine-tagged fusion proteins from bacteria. These kits have also been used for the purification of 6xHistidine-tagged proteins from baculovirus-infected insect cells. As the name suggests, six consecutive histidine residues are recombinantly fused to the protein of interest and expressed in bacteria. The resulting 6xHis fusion protein can be extracted from bacteria by using B-PER® Bacterial Protein Extraction Reagent and then purified using one of the pre-packaged Nickel Chelated Columns (Ni-Chelated Columns) or Handee™ Spin Columns included. The patented detergent in B-PER® Reagent, combined with a small amount of imidazole, efficiently washes off nonspecifically and/or weakly bound proteins (e.g., proteins rich in histidine residues). The 6xHistidine-tagged proteins are then eluted with excess imidazole (Elution Buffer).

Figure 11. SDS-PAGE analysis of the purification of 6xHis-tagged GFP from 6xHis Fusion Protein Column Purification Kit.

The kit protocol has been optimized to give a high yield of pure 6xHistidine fusion protein in the fastest amount of time. All of the buffers have been optimized for the most efficient purification. Use of other buffer formulations may significantly alter not only the binding efficiency of the column, but also the yield and/or the purity of the 6xHistidine-tagged protein. The column has been tested for loading up to 20 ml lysates from 500 ml cultures. However, for optimal results, a 10 ml lysate from 250 ml bacterial culture (OD600 = 1.5-3) is suggested as the starting material. Loading a sample size over the column capacity may cause the proteins to precipitate on the column and thus stop or hinder the flow-through. The yield and purity greatly depend on the expression level and the nature of the recombinant protein. As an example, we routinely obtain 10-12 mg of 6xHistidine-tagged green fluorescent protein (GFP) from 250 ml overnight bacterial culture with more than 90% purity using the protocol found in the kit instructions.

Ordering Information

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<td>Y-PER® Yeast Protein Extraction Reagent</td>
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For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
Fusion Protein Purification Kits

Table 4. The yield and purity of four eluents of 6xHis GFP.

<table>
<thead>
<tr>
<th>Elution</th>
<th>Yield (mg)</th>
<th>Purity (%)</th>
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<tr>
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<td>1.6</td>
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<td>2</td>
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<td>4</td>
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Table 4. The yield and purity of four eluents of 6xHis GFP.

Figure 12. Purification of 6xHis-GFP using the B-PER® Fusion Protein Spin Kit. Recombinant 6xHis-GFP expressed in E. coli BL21 was first extracted by B-PER® Reagent (Lane 1), before binding to affinity gel (Lane 2), the 6xHis-GFP-bound gels were transferred to spin columns and washed once with wash buffer to remove contamination (Lane 3). The recombinant proteins were eluted four times to achieve complete elution. Lanes 4–5 are eluant 2 and eluant 3 of 6xHis-GFP. Lane M is the molecular weight marker.

Figure 13. SDS-PAGE analysis of the GST purification using B-PER® GST Fusion Protein Column Purification Kit. Fractions from each purification step were subjected to SDS-PAGE analysis using a gradient 4%–20% SDS-PAGE gel and stained with GelCode Blue Stain Reagent. Lane 1, the crude lysate extracted from E. coli with B-PER® Reagent. Lane 2, the flow-through from the crude lysate, Lanes 3–4, wash fractions of Wash Buffer 1, Lane 5, wash fractions of Wash Buffer 2, and Lane 6, GST eluted from the column with the elution buffer (50 mM glutathione).
The development of B-PER system for GST fusion protein purification. Fusion Protein Spin Purification Kit provides a highly efficient resin was then transferred to a spin column (0.75 ml per spin column) and placed in 2 ml collection tubes. Following a brief spin, the GST protein was eluted four times with 0.3 ml of 25 mM glutathione (reduced) to achieve complete elution. The GST yield and purity are listed in Table 5.

Table 5. The yield and purity of four eluents of GST GFP.

<table>
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<tr>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<tr>
<td>Yield (%)</td>
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<td>97.0</td>
<td>99.1</td>
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<tr>
<td>Purity (%)</td>
<td>91.3</td>
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<td>97.3</td>
<td>99.4</td>
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<td>95.4</td>
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</table>

The Y-PER® 6xHis and GST Fusion Protein Column Purification Kits

High-capacity purification > 10 mg/column.

- Provides a high yield of purified protein in a short amount of time
- Buffers are optimized for the most efficient purification
- Purify 6xHis fusion protein from Saccharomyces cerevisiae, Schizosaccharomyces pombe and Bacillus subtilis

Y-PER® 6xHis Column Kit

The Y-PER® 6xHis Fusion Protein Column Purification Kit is designed for rapid and efficient purification of 6xHis fusion proteins from yeast or bacteria. As the name suggests, six consecutive histidine residues are fused to the protein of interest as the recombinant protein is expressed in yeast or bacteria. The resulting 6xHis fusion protein can be extracted using Y-PER® Yeast Protein Extraction Reagent and subsequently purified using one of the pre-packaged nickel-chelated columns provided in the kit. The proprietary wash buffers and Y-PER® Reagent efficiently separate nonspecifically and/or weakly bound proteins (e.g., proteins rich in exposed histidine residues) from the 6xHis fusion protein that has a strong affinity for the Ni-chelated column. The 6xHis fusion protein is then eluted from the column with the application of a buffer that contains a high concentration of imidazole (Elution Buffer). All contents of this kit are supplied ready to use.

Fresh Cells and Frozen Cells:

- Y-PER® Reagent is capable of extracting proteins equally well from both freshly harvested and previously frozen cells.

Cell Density and Strain Variation:

Differences in organism, media, strain genotype and growth conditions can have dramatic effects on the yield of cells obtained from a given volume of culture. Following are several suggestions for the volume of Y-PER® Reagent to add for a given mass of wet cell paste.

Saccharomyces cerevisiae: Y-PER® Reagent works equally well on cells grown to saturation or cells isolated from log-phase growth in both rich or synthetic defined media. Pierce recommends using 2.5-5.0 ml of Y-PER® Reagent for a 1 g cell pellet, which can be scaled up or down accordingly.

Schizosaccharomyces pombe: Y-PER® Reagent works best on cells grown in media such as Edinburgh Minimal Media (EMM). To achieve adequate lysis of cells grown in rich media such as YES, they must be harvested during log-phase growth. Pierce recommends using 2.5-5.0 ml of Y-PER® Reagent for a 1 g cell pellet, which can be scaled up or down accordingly.

Note: For cultures of S. pombe grown past log-phase, increased incubation temperature (40°C) and the addition of protease inhibitors to Y-PER® Reagent has been shown to increase lysis efficiency.

Bacillus subtilis: Y-PER® Reagent will not lye B. subtilis spores. When using a strain that is able to sporulate, take care to harvest the cells during log-phase growth. For strains unable to sporulate, cells can be grown to saturation prior to lysis. Pierce recommends using 2.5-5.0 ml of Y-PER® Reagent for a 1 g cell pellet, which can be scaled up or down accordingly.

Escherichia coli: Y-PER® Reagent works very well on E. coli. Pierce recommends using 2.5-5.0 ml of Y-PER® Reagent for a 1 g cell pellet, which can be scaled up or down accordingly.
Fusion Protein Purification Kits

Y-PER® 6xHis and GST Fusion Protein Column Purification Kits

Y-PER® GST Fusion Protein Column Purification Kit is designed for rapid and efficient purification of glutathione S-transferase (GST) fusion proteins from yeast or bacteria. Expressed GST fusion proteins can be extracted using Y-PER® Yeast Protein Extraction Reagent, and subsequently purified using one of the pre-packaged Immobilized Glutathione Columns provided in the kit. The proprietary wash buffers and Y-PER® Reagent efficiently separate nonspecifically and/or weakly bound proteins from the GST fusion protein that has a strong affinity for the Immobilized Glutathione Column. The GST fusion protein is then eluted from the column and recovered in a highly purified form with the application of a buffer that contains a high concentration of reduced glutathione.

Ordering Information

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<tr>
<td>78594 Y-PER® 6xHis Fused Protein Column Purification Kit</td>
<td>Kit</td>
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<tr>
<td>Contains sufficient reagents for five 6xHis fusion protein purifications from Staphylococcus aureus, Shigella sonnei, Pasteurella multocida, Escherichia coli, or Streptococcus equisimilis.</td>
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<tr>
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<td>Buffer A</td>
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<td>78797 Y-PER® GST Fusion Protein Column Purification Kit</td>
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<tr>
<td>Contains sufficient reagents for five GST fusion protein purifications from Escherichia coli.</td>
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<td>Includes: Y-PER® Yeast Protein Extraction Reagent</td>
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<td>Wash Buffer 1</td>
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β-Galactosidase Assay Kits

All-in-One™ Mammalian β-Galactosidase Assay Kit

Single reagent for cell- or lysate-based assays.

Highlights:
- One-reagent formula for cell- or lysate-based assays
- Fast and efficient lysis with liquid reagents
- No harvesting or washing steps
- Flexible protocols for different size cultures

Comparison of Pierce All-in-One™ β-Gal Assay Kit with Brand P β-Gal Assay Kit.

Pierce All-in-One™ β-Gal Assay Kit
1. Remove media from plate.
2. Add All-in-One™ Assay Reagent and incubate at 37°C for 30 minutes.
3. Stop is optional.
4. Measure absorbance at 405 nm.

Brand P β-Gal Assay Kit
1. Remove media from plate.
2. Wash cells twice with PBS.
3. Dilute 0.5X Reporter Lysis Buffer to 1X.
4. Add Reporter Lysis Buffer and incubate for 15 minutes.
5. Scrape cells.
6. Remove cells to a clean tube, vortex and centrifuge for 2 minutes at 4°C for 30 minutes.
7. Add 2X Assay Buffer and incubate at 37°C for 30 minutes.
8. Stop the reaction.
9. Measure absorbance at 405 nm.

Advantages Offered by the Pierce All-in-One™ β-Gal Assay Kit:
1. The Pierce All-in-One™ β-Gal Assay Kit saves time. The average researcher would carry out a four-step procedure with the Pierce system as opposed to a nine-step procedure with the Brand P system.
2. The Pierce All-in-One™ β-Gal Assay Kit contains the active ingredient M-PER® Mammalian Protein Extraction Reagent, which allows extraction of 60% more protein than Brand P’s cell lysis reagent. More β-Gal activity can be detected with the Pierce All-in-One™ β-Gal Assay Kit because cells are lysed efficiently.

Ordering Information

<table>
<thead>
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<th>Product # Description</th>
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<tr>
<td>75706 All-in-One™ Mammalian β-Galactosidase Assay Reagent</td>
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<tr>
<td>75707 All-in-One™ Mammalian β-Galactosidase Assay Kit</td>
<td>Kit</td>
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<td>Includes: All-in-One™ Mammalian β-Galactosidase Assay Reagent</td>
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<td>M-PER® Mammalian Protein Extraction Reagent</td>
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<td>Stop Solution</td>
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</table>

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
β-Galactosidase Assay Kit

Ideal for identifying protein:protein interactions in vivo using two-hybrid systems.

Highlights:
• Efficiently fuses yeast cells and a colorimetric detection system
• Quantitative or qualitative assay
• Allows researcher to test cell cultures directly with no harvesting and washing steps (ideal for screening applications)
• Assay activity from colonies growing on solid media, qualitative or quantitative, with no re-streaking involved
• Can be used with bacterial cells

The gene encoding β-Galactosidase (α2β) of E. coli has been widely used as a reporter gene in many different prokaryotic and eukaryotic organisms. In particular, this gene has proven useful for studying gene expression in the yeast S. cerevisiae.

In addition to its utility in studying the regulation of gene expression, the measurement of β-Galactosidase activity can be used to identify protein:protein interactions in vivo using two-hybrid systems. The strength of the interaction is usually verified and/or quantitated for studying gene expression in the yeast S. cerevisiae.

In contrast to methods using 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) as a β-Galactosidase substrate, our reagent system allows for the qualitative or quantitative determination of β-Galactosidase activity in solution directly from colonies growing on solid medium. Part of a colony is picked from a plate and resuspended in a mixture of 1 x 10⁶ β-Yeast Protein Extraction Reagent and 1 x 10⁶ β-Galactosidase assoy buffer. After a brief incubation period, the solution turns yellow from the hydrolysis of 4-nitrophenyl-β-D-galactopyranoside (ONPG) to 4-nitrophenol (ONP) and galactose in a mildly alkaline solution. The assay becomes quantitative if the quantity of cells in the assay is first determined with an absorbance reading taken at 660 nm (OD₆₆₀).

β-Galactosidase activity proceeds until two clear phases are formed where proteins partition according to their hydrophilic and hydrophobic features. Membrane proteins are enriched in the hydrophobic fraction. Pierce uses this partitioning method in the Mem-PER® Eukaryotic Membrane Protein Extraction Kit (Product #89826). The protocol involves the isolation of integral membrane proteins and proteins via phase partitioning at a physiological temperature.2

Typical cross-contamination is ≤10%; Figure 22). With this stepwise fractionation procedure, concentrated nuclear extracts are obtained and gene regulation experiments are not compromised, as is common when seen whole cell lysates are analyzed. Prepared extracts are compatible with many downstream applications, including electrophoretic mobility shift assays (EMSA) with nuclear extracts, reporter assays with cytosolic extracts, Western blots, enzyme assays, and BCA™ Protein Assays.

Membrane Proteins
Membrane proteins constitute approximately 30% of the eukaryotic proteome3 and are a key target in drug discovery research. However, they are difficult to isolate because of their hydrophobicity, basic nature and large size.

Triton® X-114 is a unique detergent that is used to not only solubilize membrane proteins, but to also separate them from hydrophobic proteins via phase partitioning at a physiological temperature.2

Solutions of Triton® X-114 are homogeneous at 0°C (form a clear micellar solution) but separate aqueous and detergent phases above 20°C (the cloud point) as micellar aggregates form and the solution turns turbid. With increased temperature, phase separation proceeds until two clear phases are formed where proteins partition according to their hydrophilic and hydrophobic features. Membrane proteins are enriched in the hydrophobic fraction. Pierce uses this partitioning method in the Mem-PER® Eukaryotic Membrane Protein Extraction Kit (Product #89826). The protocol involves the gentle lysis of mammalian cells using a mild, proprietary detergent followed by membrane protein extraction using the nonionic detergent Triton® X-114. Yeast cells can be fractionated as well using the Mem-PER® Kit in combination with glass beads.

Cell Fractionation with Detergents
Cell fractionation and protein enrichment are important methods in the rapidly growing field of proteomics. Isolation of subcellular fractions and concentration of proteins in low abundance allow for more efficient identification and study of proteins of interest, including the isolation of integral membrane proteins and nuclear proteins.

Mitochondria Isolation and Protein Extraction
Isolation of mitochondria is typically a laborious process requiring single-sample processing with Dounce homogenization. The Mitochondria Isolation Kit for Cultured Mammalian Cells (Product #90824) uses a non-mechanical, reagent-based method for mitochondria isolation that allows multiple samples (six) to be processed concurrently. Cultured mammalian cell pellets are gently lysed using a proprietary formulation (patent pending) that results in maximum yield of mitochondria with minimal damage to integrity. The product instructions describe guidelines for optimizing purity vs. yield parameters. The kit also offers an optimized Dounce homogenization procedure, which results in recovery of twice as much mitochondria (measured as mitochondrial protein) than the reagent-based method. Both methods use differential centrifugation to separate the mitochondrial and cytosolic fractions with a bench-top microcentrifuge and are completed in approximately 40 minutes (post-cell harvest). Once isolated, the mitochondria can be used in downstream applications such as apoptosis, signal transduction and metabolic studies, as well as to facilitate mitochondrial proteomics efforts.

Nuclear Proteins
Designed for the stepwise isolation of nuclear and cytoplasmic proteins using a mild detergent, the NE-PER® Nuclear and Cytoplasmic Extraction Reagents (Product #78833) prepare extracts from cultured mammalian cells and tissues by first disrupting the outer cell membrane to obtain the cytoplasmic contents and then extracting proteins from the nucleus. Cross-contamination between the two fractions is minimal (<10%). With this stepwise fractionation procedure, concentrated nuclear extracts are obtained and gene regulation experiments are not compromised, as is common when seen whole cell lysates are analyzed. Prepared extracts are compatible with many downstream applications, including electrophoretic mobility shift assays (EMSA) with nuclear extracts, reporter assays with cytosolic extracts, Western blots, enzyme assays, and BCA™ Protein Assays.

References

Ordering Information

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<td>2 x β-Galactosidase Assay Buffer</td>
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<td>1 M Na₂CO₃ Stop Solution</td>
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</table>

Figure 21. Reproducibility of β-Galactosidase assay using the novel lysis reagent. The average of three serial dilutions from two independent experiments performed weeks apart demonstrates the reproducibility of the assay. In both experiments, cells were grown to an OD₆₆₀ of 0.7, then 100 μL of cells were transferred to a 96-well plate and serially diluted into fresh media. β-Galactosidase activity was determined after 20 minutes (unit=OD₄₀₅ x 1,000/time/OD₆₆₀). In data not shown, Pierce researchers see good reproducibility and the similar specific activity even down to 60,000 cells/well with this expression level. Weaker expression was observed in more cells.

Figure 20. Linearity of β-Galactosidase assay from cells growing in media in a 96-well plate. Strept PK1202 carrying plasmid pYX125-β-Gal was grown to an OD₆₆₀ of 1.0, then 100 μL of cells were transferred to a 96-well plate. At time 0, 100 μL of a 1:1 mixture of lysos magnet and 2X β-gal assay buffer were added to each well and absorbance at 405 nm was determined. Specific activity for this sample is 160 units (unit=OD₄₀₅ x 1,000/time/OD₆₆₀).

Figure 22. The NE-PER® Reagent’s stepwise extraction process results in minimal cross-contamination between cytoplasmic and nuclear fractions. Oct-1 and Hüp50 determined by Western blot analysis. β-Gal determined by activity assay. Typical cross-contamination is ≤10%.
Mem-PER® Eukaryotic Membrane Protein Extraction Kit

Efficient, gentle reagents that solubilize and isolate membrane proteins in one hour!

Mem-PER® Reagents and 2-D Sample Prep for Membrane Proteins expand to include hard and soft mammalian tissues.

**Highlights:**
- Includes an easy and complete protocol that allows for the isolation of membrane proteins in approximately one hour
- Introduces minimal cross-contamination (typically <10%) of hydrophilic proteins into the hydrophobic (membrane protein) fraction
- Works with a variety of hard and soft tissues (Figures 23A and 24)
- Works with a variety of eukaryotic cell types (e.g., mammalian (Figure 25) and yeast systems (Figure 26))
- Provides compatibility with downstream applications including SDS-PAGE, Western blotting, BCA® Assays, etc. (use of Pierce Slide-A-Lyzer™ Dialysis Products and the PAGEprep™ Advance Kit (Product # 89888) can aid these procedures)

**Isolation of membrane proteins can be a tedious, time-consuming process requiring gradient methods and expensive ultracentrifuge equipment** that can be cumbersome and produce poor protein yields. Ideally, the isolation process should be mild, yet rapid. Recognizing that detergents provide a more convenient method for extraction,4 Pierce researchers developed the Mem-PER® Eukaryotic Membrane Protein Extraction Kit, a faster, easier and less expensive way to isolate membrane proteins. The Mem-PER® Kit consists of three reagents developed for the enrichment of integral membrane proteins obtained from cultured eukaryotic cells and tissues.

The simple protocol is accomplished in two parts (Figure 23B). First, cells are lysed with a proprietary detergent and then a second proprietary detergent is added to solubilize the membrane proteins. Second, the hydrophobic proteins are separated from the hydrophilic proteins through phase-partitioning.4 Following careful separation of the two layers, membrane proteins are ready for subsequent yields. Ideally, the isolation process should be mild, yet rapid.

**Figure 24. Western blots of membrane protein extract prepared from rat liver (panel A) and rat heart (panel B).** Hydrophobic (membrane protein) fractions and corresponding hydrophilic fractions prepared with Mem-PER® Reagents were analyzed by Western blot for VDAC (31 kDa, affinity BioReagents), Cellubin (29 kDa, BD Transduction Laboratories) and Flotillin-1 (44 kDa, BD Transduction Laboratories). Mit: membrane fraction and H: hydrophilic fraction.

The Mem-PER® Kit is compatible with many downstream applications. The isolated membrane protein fraction can be used directly in SDS-PAGE and Western blotting (Figure 25). BCA® Assays, subsequent purification, etc. can be performed following removal of Reagent C through dialysis using convenient Pierce Slide-A-Lyzer™ MINI Dialysis Units or Slide-A-Lyzer™ Dialysis Cassettes. To effectively remove Reagent C and simultaneously maintain protein solubility, perform dialysis overnight at 4°C with a buffer that includes 0.5% detergent. Quantification of extracted membrane proteins with the Pierce Micro BCA® Protein Assay Reagent Kit typically results in approximately 250 µg of total protein from 5 x 10^6 C6 cells. The total amount of protein obtained will vary with cell line.

**Figure 25. Partitioning of solubilized proteins using Mem-PER® Reagent.** Proteins from three cell lines were solubilized and extracted using the Mem-PER® Kit. Each set of hydrophobic and hydrophilic (membrane protein) fractions obtained were normalized to one another and analyzed by Western blot for four proteins from the cellular fractions noted. The Pierce® Protein Clean-up and Enrichment Kit was used to remove the detergent from the membrane fraction before SDS-PAGE/Western analysis of CoxA, VDAC, Cellubin, and Flotillin-1. Membrane protein bands were found in all debris fractions. Abbreviations: AchE = acetylcholinesterase, CoxA = cytchrome oxidase subunit 4, hsp90 = heat shock protein 90, M = solubilized membrane protein fraction and H = hydrophilic protein fraction.

**Figure 26. Use of the Mem-PER® Kit to solubilize and isolate yeast integral membrane proteins.** (A) Achromatococcus caryophyllaceus strain EY-193 (29 kDa, BD Transduction Laboratories). M: membrane protein fraction and H: hydrophilic fraction. The isolated membrane protein fraction can be used directly in SDS-PAGE. (B) Achromatococcus caryophyllaceus strain EY-194. Approximately 250 µg of total protein from 5 x 10^6 C6 cells. The total amount of protein obtained will vary with cell line.

**References**

**Ordering Information**

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<td>Kit</td>
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<td>69964</td>
<td>2-D Sample Prep for Membrane Proteins</td>
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<td>78417</td>
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Visit [www.piercenet.com](http://www.piercenet.com) for 2-D Sample Preparation Applications. Pierce has incorporated Mem-PER® Reagents into a new kit (Product # 89884, 2-D Sample Prep for Membrane Proteins) that isolates, concentrates and cleans up membrane proteins.

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
Mitochondria Isolation Kit for Cultured Cells

Isolate intact mitochondria with maximum yield in only 49 minutes.

Highlights:
- Fast – isolate intact mitochondria in approximately 40 minutes
- Multi-sample format – reagent-based method allows for concurrent preparation of multiple samples
- Optional alternate method – reagents and protocol included for traditional Dounce homogenization
- Benchtop compatibility – isolation performed in a microcentrifuge tube

The isolation of mitochondria is typically a laborious process requiring single-sample processing with Dounce homogenization. The new Mitochondria Isolation Kit from Pierce uses a non-mechanical, reagent-based method (Figure 27A) that allows multiple samples (<6) to be processed concurrently. Cultured mammalian cell pellets are gently lysed using a proprietary formulation that results in maximum yield of mitochondria with minimal damage to integrity. The kit also offers a second isolation method based on traditional Dounce homogenization (Figure 27B), which results in two-fold more mitochondria recovery, as determined by protein assay. Both methods use differential centrifugation to separate the mitochondrial and cytosolic fractions with a benchtop microcentrifuge and are completed in approximately 40 minutes (post-cell harvest). Once isolated, the mitochondria can be used in downstream applications such as apoptosis, signal transduction and metabolic studies, as well as to facilitate mitochondrial proteomics efforts.

Mammalian cells (2 x 10⁷)

Mitochondria isolation

The reagent- and Dounce-based isolation procedures are outlined in Figure 27A and 27B, respectively.

Approximately 2 x 10⁷ mammalian cells (NIH3T3 or C6) were pelleted per sample in a 2 ml microcentrifuge tube. The cells were resuspended in Mitochondria Isolation Reagent A and incubated on ice for 2 minutes. Using the reagent-based method, the cells were lysed by adding Mitochondria Isolation Reagent B in conjunction with frequent vortexing. The lysate was mixed with Mitochondria Isolation Reagent C and centrifuged to remove nuclei, unbroken cells and cellular debris. The supernatant was subsequently centrifuged to collect the mitochondria, and the pellet was surface-washed to remove cytosolic contaminants. Mitochondria prepared using Dounce homogenization followed a similar protocol. Briefly, 2 x 10⁷ NIH3T3 cells were resuspended in Mitochondria Isolation Reagent A, incubated for 2 minutes and then transferred to a tissue grinder and lysed with 80 strokes. The cell homogenate was mixed with Mitochondria Isolation Reagent C and the remainder of the protocol detailed in Figure 27 was performed.

Damage to the outer membrane was assessed by Western blot analysis of Cytochrome C and voltage-dependent anion channel (VDAC) (Figure 28). Cytochrome C resides in the intermembrane space of undamaged mitochondria and VDAC is an integral membrane protein in the outer mitochondrial membrane. Negligible amounts of both proteins were present in the cytosol, indicating that the mitochondria remained intact during isolation. Contamination of the mitochondria with cytosolic components was found to be negligible. Following a single wash of the collected pellet of mitochondria, minimal heat shock protein 90 (HSP90) contamination was detected in a Western blot (Figure 29).

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007

www.piercenet.com/po/p5d • E-mail Customer Service: CS@piercenet.com

Cell Fractionation

Mitochondria Isolation Kit for Tissue

The Mitochondria Isolation Kit for Tissue offers two methods for the isolation of intact mitochondria from both soft and hard tissues. The first method uses a unique reagent-based procedure that enables simultaneous multi-sample processing. The second method relies on traditional Dounce homogenization for tissue disruption and subsequent isolation of the organelle. Both procedures rely on differential centrifugation to separate the intact mitochondria using a bench-top microcentrifuge and are completed in less than 60 minutes. Both procedures have been optimized for maximum yield of mitochondria with minimal damage to the integrity of the organelle. The isolated mitochondia may be used for a number of downstream applications, including 2D-Maldi for proteomics research, disease profiling and metabolic studies.

References for Mitochondrial Proteins

### Table 6. Collection of mitochondria (reagent-based method).

<table>
<thead>
<tr>
<th>Protein Location</th>
<th>Protein (µg)</th>
<th>% Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>155</td>
<td>58</td>
</tr>
<tr>
<td>M1</td>
<td>12,000</td>
<td>59</td>
</tr>
<tr>
<td>M2</td>
<td>200</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>12</td>
</tr>
</tbody>
</table>

Mitochondria collection, normally performed at 12,000 g, was split into a low-speed collection at 3,000 x g and a subsequent high-speed collection of remaining mitochondria in the supernatant at 12,000 x g.

The isolated mitochondia may be used in many downstream applications, including Western blotting. 2-D Western analysis of isolated mitochondia was used to identify Mn-Superoxide Dismutase, a detoxifying enzyme residing in the mitochondrial matrix (Figure 31).

Figure 31. 2-D Western blot of superoxide dismutase (Mn-SOD) in isolated mitochondria. Mitochondria were isolated from NIH3T3 cells using the Dounce method, resolved by 2-D gel and analyzed by Western blot for manganese-containing SOD. Approximately 15 µg of mitochondrial protein was focused on an 11 cm, pH 5-10 IPG strip. The second dimension was performed by 8% SDS-PAGE.

Ordering Information

<table>
<thead>
<tr>
<th>Product #</th>
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<tbody>
<tr>
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<tr>
<td></td>
<td>Kit</td>
</tr>
<tr>
<td>89681</td>
<td>Mitochondria Isolation Kit for Tissue</td>
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<tr>
<td></td>
<td>Kit</td>
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</table>

**See Table 6 for protocol quantification.

<table>
<thead>
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<th>Protein</th>
<th>Location</th>
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</tr>
<tr>
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<td>59</td>
<td></td>
</tr>
<tr>
<td>M2</td>
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<td>10</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>12</td>
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</tr>
</tbody>
</table>

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<tr>
<td></td>
<td>Kit</td>
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<td>89681</td>
<td>Mitochondria Isolation Kit for Tissue</td>
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<td></td>
<td>Kit</td>
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</table>

**See Table 6 for protein quantification.

<table>
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<th>Protein (µg)</th>
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</thead>
<tbody>
<tr>
<td>M</td>
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<td>58</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>12,000</td>
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<tr>
<td>M2</td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>
Cell Fractionation

Figure 32 shows the results of Micro BCA and Cytoplasmic Extraction Reagents, which enable a stepwise lysis of cell lines from a variety of mammalian cell lines using NE-PER. Most of these are, however, lengthy processes affected by cellular components present in whole cell lysates. The preparation of good nuclear protein extracts is central to the success of many gene regulation studies. Nuclear extracts are used instead of whole cell lysates for the following reasons. First, the yield of protein in the nuclear extract is more easily by varying the volume of nuclear extraction reagent (NER) used in the extraction without any significant loss in extraction efficiency. Preparation of a cytoplasmic and nuclear fractions, so it is important to maintain the integrity of the two cellular compartments. Western blot analyses were used to assess the level of cross-contamination between the two fractions (Figure 33).

The data show that the yield of protein in the cytoplasmic extract is cell-line dependent, with the larger cell sizes such as HeLa and Hepa 1-6 giving more total protein (350 µg) compared to the smaller NIH3T3 fibroblasts and rat C6 brain cells (250 µg). Nuclear protein yields averaged 100-200 µg total protein from 2 x 10^6 cells at a concentration on the order of 1.5 mg/ml, independent of cell type. The concentration of nuclear proteins can be manipulated easily by varying the volume of nuclear extraction reagent (NER) used in the extraction without any significant loss in extraction efficiency. The key to success for the NE-PER Kit is the stepwise isolation of cytoplasmic and nuclear fractions, which is so important to maintain the integrity of the two cell compartments. Western blot analyses of cells that generates both functional cytoplasmic and nuclear protein fractions in less than two hours. Figure 32 shows the results of Micro BCA Protein Assays performed on both the cytosolic and nuclear fractions prepared from a variety of cell lines.

Figure 33. Western blot analyses of HeLa cytoplasmic and nuclear fractions illustrating the low level of cross-contamination. Four samples were analyzed by loading equivalent amounts of total protein (20 µg) in each lane of a 4-20% gradient Tris-Glycine denaturing gel. The protein was transferred to nitrocellulose and blocked with 3% BSA in Tris buffered saline. A) Blot probed with Oct-1 antiserum (1:400) followed by a 1:100,000 secondary antibody-horseradish peroxidase (HRP) conjugate. B) Blot probed with HRP anti-human α-actin antiserum (1:400) followed by a 1:5,000 secondary antibody-HRP conjugate. Both blots were developed using Pierce SuperSignal® West Pico Chemiluminescent Substrate (Product # 34080).

Figure 34. Compartmentalization of cytoplasmic and nuclear fractions is maintained using NE-PER Reagents. A) Extracts from C6 cells (rat glial cells expressing β-galactosidase) were assayed for β-Gal activity using a commercially available kit. B) DNA polymerase activity was assayed using primed M13 single-stranded DNA, buffer, salt and nucleotide composition obtained from the literature. DNA synthesis was monitored by quantifying the amount of [3H]-dCTP incorporated into TCA-precipitable cpm.

The electrophoretic mobility shift assay (EMSA) is one of the key applications in the area of gene regulation. Nuclear extracts prepared with NE-PER® Reagents were used with the LightShift™ Chemiluminescent EMSA Kit (Product #20148).

The volume of nuclear extract used in these reactions did not exceed 2 µl (10% of total reaction volume). If the protein of interest is less abundant, the concentration of the nuclear extract can be increased by decreasing the volume of NE-PER® Extraction Reagent used during extraction. If it still is necessary to use larger volumes of extract for an EMSA, Pierce Oleo-A™ MINI Dialysis Units can be used to remove interfering substances in the NER Nuclear Reagent.

References

Ordering Information

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<thead>
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<td>NE-PER® Nuclear and Cytoplasmic Extraction Kit</td>
<td>Kit</td>
</tr>
<tr>
<td>89633</td>
<td>2-D Sample Prep for Nuclear Proteins</td>
<td>Kit</td>
</tr>
</tbody>
</table>

For Technical or Customer Assistance, Call 800-874-3723 or Fax 804-842-5007
DNA Extraction

Y-DER® Yeast DNA Extraction Kit

Extracts and purifies genomic and plasmid DNA from yeast in less than one hour.

Highlights:
- Eliminates the need for glass beads or harsh enzyme treatments
- Prepares DNA for polymerase chain reaction (PCR) amplification
- Rapidly isolates plasmid DNA from Saccharomyces cerevisiae suitable for transformation of Escherichia coli
- Scalable kit — from single colonies to 500 ml culture

Current protocols for the extraction and purification of DNA from yeast are time-consuming and labor-intensive. The yeast cell is notoriously difficult to lyse due to a very complex proteinaceous cell wall that provides rigidity to the relatively weak plasma membrane. The Y-DER® Reagent Kit uses Y-PER® Reagent to provide amplification-ready plasmid DNA for the PCR. The Y-DER™ Reagent kit is all one needs to lyse the cell and release the template DNA for direct PCR application. The versatility of the Lyse-N-Go™ Reagent is all one needs to lyse yeast cells.

The Y-DER™ Reagent Kit surpasses the historical methods of DNA isolation from yeast. The protocol requires less than one hour, works without enzymatic treatment or glass beads, and yields little DNA contamination.

In studies with S. cerevisiae, Y-DER™ Reagent consistently obtains high yields of genomic and plasmid DNA. Y-DER™-purified DNA is suitable for PCR amplification (Figure 36), bacterial transformations (both chemical and electroporation), restriction enzymes, and PCR amplification.

DNA Extraction

Properties and Types of Detergents

Detergents are amphiphilic molecules, meaning they contain both a nonpolar "tail" having aliphatic or aromatic character and a polar "head" (Figure 37). Ionizing groups of the polar head groups form the basis for broad classification of detergents; they may be ionic (charged, either anionic or cationic), nonionic (uncharged), or zwitterionic (having both positively and negatively charged groups but with a net charge of zero).

The components of biological membranes, detergents have hydrophobic-associating properties as a result of their nonpolar tail groups. Nevertheless, detergents are themselves water-soluble. Consequently, detergent molecules allow the dispersion (miscibility) of water-insoluble, hydrophobic compounds into aqueous media, including the extraction and solubilization of membrane proteins.

Both the number of detergent monomers per micelle (aggregation number) and the range of detergent concentration above which micelles form (called the critical micelle concentration, CMC) are properties specific to each particular detergent (Table 7, page 29). The critical micelle temperature (CMT) is the lowest temperature at which micelles can form. The CMT corresponds to what is known as the cloud point because detergent micelles form crystalline suspensions at temperatures below the CMT and are clear again at temperatures above the CMT.

Detergent properties are affected by experimental conditions such as concentration, temperature, buffer pH, and ionic strength, and the presence of various additives. For example, the CMC of certain nonionic detergents decreases with increasing temperature, while the CMC of ionic detergents decreases with addition of counter ions as a result of reduced electrostatic repulsion among the charged head groups. In other cases, additives such as urea effectively disrupt water structure and cause a decrease in detergent CMC. Generally, dramatic increases in aggregation number occur with increasing ionic strength.

Detergents can be denaturing or non-denaturing with respect to protein structure. Denaturing detergents can be anionic, such as sodium dodecyl sulfate (SDS), or cationic, such as ethyl trimethyl ammonium bromide. These detergents totally disrupt membranes and denature proteins by breaking protein-protein interaction.

Non-denaturing detergents can be divided into nonionic detergents such as Triton X-100, bile salts such as cholate, and zwitterionic detergents such as CHAPS.

Protein Binding and Solubilization

Denaturing detergents such as SDS bind to both membrane (hydrophobic) and nonmembrane (water-soluble, hydrophilic) proteins at concentrations below the CMC, i.e., monomers. The reaction is equilibrium-driven until saturated. Therefore, the free concentration of monomers determines the detergent concentration. SDS binding is cooperative (the binding of one molecule of SDS increases the probability that another molecule of SDS will bind to that protein) and alters most proteins into rigid rods whose length is proportional to molecular weight.

Non-denaturing detergents such as Triton X-100 have rigid and bulky nonpolar heads that do not penetrate into water-soluble proteins; consequently, they generally do not disrupt native interactions and structures of water-soluble proteins and do not have cooperative binding properties. The main effect of non-denaturing detergents is to associate with hydrophobic parts of membrane proteins, thereby conferring miscibility to them.

At concentrations below the CMC, detergent monomers bind to water-soluble proteins. Above the CMC, binding of detergent to proteins competes with the self association of detergent molecules into micelles. Consequently, there is effectively no increase in protein-bound detergent monomers with increasing detergent concentration beyond the CMC.

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
Detergents

Detergents with low CMCs and large aggregation numbers cannot be effective for removal of detergents that have very high CMCs. In assay or gel electrophoresis, an artificial membrane (although recovery of native orientation in such cases is studied in isolation if it is first purified and then reconstituted into lipid mixtures. The function of an individual protein can be disrupted. In some such cases, membrane protein function has been disrupted. In some such cases, membrane protein function can be restored when they are reconstituted into bilayers membranes by replacement of detergent with phospholipids or other membrane-like lipid mixtures. The function of an individual protein can be studied in isolation if it is first purified and then reconstituted into an artificial membrane (although recovery of native orientation in the membrane is a major challenge). Even where restoration of protein function is not an issue, detergent concentration may have to be decreased in a sample to make it compatible with protein assays or gel electrophoresis.

Removal of Detergent from Solubilized Proteins

However necessary and beneficial the use of detergent may have been for initial cell lysis or membrane protein extractions, subsequent applications or experiments with the extracted proteins may require removal of some or all of the detergent. For example, although many water-soluble proteins are functional in detergent-solubilized form, membrane proteins are often modified and inactivated by detergent solubilization as a result of native lipid interactions having been disrupted. In some such cases, membrane protein function is restored when they are reconstituted into bilayers membranes by replacement of detergent with phospholipids or other membrane-like lipid mixtures. The function of an individual protein can be studied in isolation if it is first purified and then reconstituted into an artificial membrane (although recovery of native orientation in the membrane is a major challenge). Even where restoration of protein function is not an issue, detergent concentration may have to be decreased in a sample to make it compatible with protein assays or gel electrophoresis.

Detergent removal can be attempted in a number ways. Dialysis is effective for removal of detergents that have very high CMCs and small aggregation numbers, such as the N-oligosaccharides. Detergents with low CMCs and large aggregation numbers cannot be dialyzed since most of the detergent molecules will be in micelles.

Table 7. Properties of common detergents.

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Description</th>
<th>Aggregation Number</th>
<th>Micelle MW</th>
<th>MW</th>
<th>CMC (mM)</th>
<th>CMC % w/v</th>
<th>Cloud Point (°C)</th>
<th>Dialyzable</th>
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</thead>
<tbody>
<tr>
<td>Triton X-100</td>
<td>Nonionic</td>
<td>140</td>
<td>90,000</td>
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<td>0.24</td>
<td>0.0155</td>
<td>64</td>
<td>No</td>
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<tr>
<td>NP-40</td>
<td>Nonionic</td>
<td>149</td>
<td>90,000</td>
<td>617</td>
<td>0.29</td>
<td>0.0179</td>
<td>80</td>
<td>No</td>
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<td>Brij-35</td>
<td>Nonionic</td>
<td>40</td>
<td>49,000</td>
<td>1225</td>
<td>0.29</td>
<td>0.1103</td>
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<td>No</td>
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<tr>
<td>Brij-58</td>
<td>Nonionic</td>
<td>15</td>
<td>62,000</td>
<td>1120</td>
<td>0.077</td>
<td>0.0266</td>
<td>&gt;100</td>
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<tr>
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<td>Nonionic</td>
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<td>—</td>
<td>1224</td>
<td>0.26</td>
<td>0.0794</td>
<td>95</td>
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<tr>
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<td>octyl glucoside</td>
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<td>0.0876</td>
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<td>—</td>
<td>—</td>
<td>308</td>
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<td>0.2772</td>
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<td>0.178-2.034</td>
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<tr>
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<td>6,149</td>
<td>615</td>
<td>8-10</td>
<td>0.490-0.575</td>
<td>&gt;100</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Surface-Amp® Purified Detergent Solutions

Nonionic detergents – ready-to-use solutions. Nothing could be easier … nothing protects better!

Highlights:

- Precise 10% detergent solutions in ultrapure water
- Easy to accurately dispense and dilute for use
- Exceptionally pure – less than 1.0 µg/mL peroxides and carbonyls
- Maximal stability and long shelf-life – packed in glass ampules under inert nitrogen gas

Surface-Pak® Detergent Sampler and Surface-Amp® Detergents

Convenient 1-sample package of detergents allows for trial testing and experimentation.

Table of Detergents

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pag.</th>
<th>Size</th>
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<td>Surface-Amp® Purified Detergents</td>
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<td>Surface-Amp® MP-40</td>
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<td>28350</td>
<td>Surface-Amp® X-100</td>
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<td>28352</td>
<td>Surface-Amp® X-20</td>
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<td>10</td>
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<tr>
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<td>CHAPS</td>
<td>100 mg</td>
<td>100</td>
</tr>
<tr>
<td>28356</td>
<td>CHAPS</td>
<td>100 mg</td>
<td>100</td>
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</table>

References


Don’t begin your experiment with detergents of unknown age and purity! Surface-Amp® Purified Detergent Solutions provide unsurpassed purity, quality and stability in convenient 10% solutions. Unlike neat detergent formulations, Surface-Amps® 10% Solutions are not so viscous that you cannot aliquot them accurately. Just open an ampule and dilute the contents into your buffer at the desired concentration.
Detergents

CHAPS & CHAPSO

Zwitterionic detergents ideal for protecting the native state of proteins.

**Highlights:**
- Can be used when renaturation after SDS-PAGE is required (if gels are treated according to the procedure of Blank, et al. to remove C12 and C14 alkyl sulfates).†
- Reduced aggregation of proteins
- 2,000-5,000 fold lower in toxicity than other detergents
- Rapid and efficient removal

**Ordering Information**

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>28300 CHAPS</td>
<td>CHAPS (3-(3-Cholamidopropyl)dimethylammonio)1-propanesulfonate</td>
<td>5 g</td>
</tr>
<tr>
<td>28301 CHAPSO</td>
<td>CHAPSO (3-(3-Cholamidopropyl)dimethylammonio)-2-hydroxy-1-propanesulfonate</td>
<td>5 g</td>
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</tbody>
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SDS (Sodium dodecyl sulfate, lauryl)

The ideal detergent when resolution is most important.

**Highlights:**
- Unique distribution of carbon chain lengths in Pierce SDS (lauryl) is advantageous in resolving viral proteins during gel electrophoresis
- Can be used when renaturation after SDS-PAGE is required (if gels are treated according to the procedure of Blank, et al. to remove C12 and C14 alkyl sulfates).†

**Ordering Information**

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<tr>
<th>Product #</th>
<th>Description</th>
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<tr>
<td>28304 SDS</td>
<td>Sodium dodecyl sulfate, laurel</td>
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Octyl β-Glucoside

A nonionic detergent widely used for membrane protein solubilization.

**Highlights:**
- Offers specific stability over Octyl
- Offers solubilizing power comparable to Octyl
- Low molecular weight permits easy removal by dialysis
- Easily removed by dialysis

**Ordering Information**

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
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<td>28302 Octyl</td>
<td>Octyl</td>
<td>5 g</td>
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<tr>
<td>28310 Octyl</td>
<td>Octyl</td>
<td>5 g</td>
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Octyl β-Thioglucopyranoside

A zwitterionic detergent that offers specific stability over Octyl β-Glucoside.

**Highlights:**
- Not affected by β-glucosidase, which is an enzyme found in some biological systems
- Optically transparent and dialyzable
- Offers solubilizing power comparable to Octyl β-Glucoside, with increased stability

**Ordering Information**

<table>
<thead>
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<th>Product #</th>
<th>Description</th>
<th>Pkg. Size</th>
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<tbody>
<tr>
<td>28351 Octyl</td>
<td>Octyl β-Thioglucopyranoside</td>
<td>5 g</td>
</tr>
</tbody>
</table>

SDS-Out” Sodium Dodecyl Sulfate Precipitation Reagent

The solution to the small-sample SDS removal problem.

**Highlights:**
- Convenient removal of SDS from microliter-volume samples
- Use for samples containing 0.1-1% SDS
- Minimal dilution of small-volume samples
- Protein sample recovery in 90%-100% range tested for proteins such as BSA, cytochrome C, soybean trypsin inhibitor, ovalbumin, ribonuclease A, myoglobin and human IgG
- Kit includes spin cup and collection tube accessories for use with the SDS-Out” SDS Precipitation Reagent

**Ordering Information**

<table>
<thead>
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<th>Product #</th>
<th>Description</th>
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<td>25208 SDS-Out” D Detergent Removing Gel</td>
<td>SDS-Out” D Detergent Removing Gel</td>
<td>10 ml</td>
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<tr>
<td>25209 SDS-Out” D Detergent Removing Gel (Pre-packed Columns)</td>
<td>SDS-Out” D Detergent Removing Gel (Pre-packed Columns)</td>
<td>5 x 1 ml</td>
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</table>

**Table 7. Detergent binding data.**

<table>
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<th>Detergent</th>
<th>Product #</th>
<th>Capacity</th>
<th>Binding Conditions</th>
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</thead>
<tbody>
<tr>
<td>Triton X-100</td>
<td>28314</td>
<td>97</td>
<td>100 mM Phosphate Buffer, pH 7.0</td>
</tr>
<tr>
<td>Tween™ 20</td>
<td>28320</td>
<td>74</td>
<td>100 mM Phosphate Buffer, pH 7.0</td>
</tr>
<tr>
<td>SDS</td>
<td>28312</td>
<td>80</td>
<td>0.05 M Tris Buffer, pH 9.0</td>
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<tr>
<td>CHAPS</td>
<td>28330</td>
<td>50</td>
<td>0.05 M Tris Buffer, pH 9.0</td>
</tr>
<tr>
<td>CHAPSO</td>
<td>28365</td>
<td>80</td>
<td>100 mM Phosphate Buffer, pH 7.0</td>
</tr>
<tr>
<td>Tris™</td>
<td>28316</td>
<td>10</td>
<td>100 mM Phosphate Buffer, pH 7.0</td>
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</tbody>
</table>

**Table 8. Protein recovery after removal of SDS from protein solution with SDS-Out” SDS Precipitation Reagent.**

<table>
<thead>
<tr>
<th>Protein*</th>
<th>Initial Concentration of SDS</th>
<th>% Protein Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bovine Serum Albumin</strong></td>
<td>97.8</td>
<td>98.0</td>
</tr>
<tr>
<td><strong>Cytochrome C</strong></td>
<td>98.9</td>
<td>99.8</td>
</tr>
<tr>
<td><strong>Soybean Trypsin Inhibitor</strong></td>
<td>98.4</td>
<td>99.2</td>
</tr>
<tr>
<td><strong>Ovalbumin</strong></td>
<td>96.4</td>
<td>96.9</td>
</tr>
<tr>
<td><strong>Ribonuclease A</strong></td>
<td>99.0</td>
<td>99.8</td>
</tr>
<tr>
<td><strong>Amylase</strong></td>
<td>99.4</td>
<td>99.8</td>
</tr>
<tr>
<td><strong>Histone IgG</strong></td>
<td>98.7</td>
<td>98.2</td>
</tr>
</tbody>
</table>

*All samples of protein solution were treated to develop this table.
Protease Inhibitors

All living organisms contain proteolytic enzymes (proteases and peptidases). Proteases are required for a variety of cellular functions, such as cellular repair or the digestion of extracellular material. In whole cells, protease activity is tightly regulated by compartmentalization or inhibitors to prevent damage to cellular proteins. Cell lyses disturbs this regulation and proteolytic degradation of the sample becomes a concern. Therefore, addition of protease inhibitors to cell lysis buffers is often required.

Protease inhibitors are biological or chemical compounds that function by reversibly or irreversibly binding to the protease. Proteases generally belong to one of four evolutionarily distinct enzyme families based on the functional groups involved in the cleavage of the peptide bond (Table 10). Therefore, several different types of inhibitors are generally required to protect proteins from proteolysis during extraction and purification.

Although the particular suite of protease inhibitors necessary to prevent degradation of proteins of interest may vary, Halt® Protease Inhibitor Cocktails (Product # 78410 and 78415) exhibit excellent inhibition of a variety of protease activities. The cocktails are suitable for the protection of proteins during extraction from animal tissues, plant tissues, yeast or bacteria and are compatible with Poppers™ Protein Extraction Reagents. Pierce also offers the protease inhibitor PMSF (Product # 36978).

**Table 10: Families of proteases and their inhibitors.**

<table>
<thead>
<tr>
<th>Family</th>
<th>Example Enzymes</th>
<th>Inhibitors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine protease</td>
<td>Trypsin, Succi dine</td>
<td>Phenylmethylsulfonyl fluoride (PMSF)</td>
</tr>
<tr>
<td>Thiol protease</td>
<td>Papain, Collagenase</td>
<td>iodoacetate, Leupeptin</td>
</tr>
<tr>
<td>Acid protease</td>
<td>Papain</td>
<td>Pepstatin A</td>
</tr>
<tr>
<td>Metalloproteases</td>
<td>Cathepsin D, Collagenase</td>
<td>EDTA</td>
</tr>
</tbody>
</table>

*Inhibitors are not all effective at concentrations of a protein family.

**Halt® Protease Inhibitor Cocktails**

Compatible protease inhibitors for all occasions!

- Halt® Protease Inhibitor Cocktails inhibit a variety of proteases. Both ready-to-use formulations inhibit serine, cysteine and calpain proteases and metalloproteases. The EDTA-free formulation is ideal for preparing samples that will be analyzed by 2-D gel electrophoresis.

**PMSF**

Reacts with serine residues to inhibit trypsin, chymotrypsin, thrombin and papain.

**Soybean Trypsin Inhibitor, Immobilized**

For effective removal of trypsin, chymotrypsin and elastase from protein digests.

---

**Protein Refolding**

Pro-Matrix® Protein Refolding Kit

*Bring your protein back into the fold!*

**Highlights:**

- Robust — conditions and components examined are limited to those having the most significant and general utility as folding buffers
- Convenient — three-level matrix design significantly reduces the amount of secondary optimization required and increases the ease of data interpretation
- Adjustable matrix format — allows refolding experiments to be customized to the target protein: proven positive and negative interactions between buffer components are addressed, minimizing unnecessary analyses
- High-purity reagents — reagents are formulated using stringent standards so that consistent results are obtained

Inclusion bodies, which consist of misfolded insoluble protein aggregates, are often the products of overexpressing recombinant proteins in bacterial hosts. Although many proteins can be refolded, it is typically a difficult and time-consuming process to create the proper conditions to restore the native conformation. The Pro-Matrix® Protein Refolding Kit is designed to simplify the development of a high-yield/high-concentration refolding protocol.

The Pro-Matrix® Protein Refolding Kit includes nine Base Refolding Buffers and seven additional buffer additives. The Base Refolding Buffers form a matrix that includes a range of strong and weak denaturant conditions for the suppression of protein aggregation (Table 11). The supplied reagents are used as additional matrix factors, depending on the protein type being refolded (Table 11 and Table 12). Buffer components are examined at three concentration levels, allowing a wide spectrum of folding conditions to be tested within one experiment. The adjustable design allows matrix conditions to be tailored to the target protein, preventing sample waste and unnecessary analysis, while maximizing refolding yields.

The Pro-Matrix® Protein Refolding Kit is accompanied by a comprehensive Refolding Guide with details on isolating, solubilizing and purifying inclusion bodies; optimizing refolding conditions; and analyzing refolding yields.

---

**Table 11: Formulation and design matrix of Pro-Matrix® Protein Refolding Kit.**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
<th>Factor 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>ilestone (IM)</td>
<td>L-Agmatine (IM)</td>
<td>Additive</td>
<td>Adenine</td>
</tr>
<tr>
<td>1</td>
<td>0.4</td>
<td>0.4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.88</td>
<td>0.88</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>5</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Ordering Information

**Product # Description**

- **89867 Pro-Matrix® Protein Refolding Kit**
  - Contains sufficient components to conduct 100 refolding experiments (1 ml each).
  - Includes: Base Refolding Buffers 1-9 (71 refolding experiments (1 ml each)).
  - Includes: Base Refolding Buffers 1-9 (71 refolding experiments (1 ml each)).
  - Includes: Base Refolding Buffers 1-9 (71 refolding experiments (1 ml each)).
  - **$675**

---

**Table 12: Buffer additives.**

<table>
<thead>
<tr>
<th>Buffer Additions</th>
<th>Amount</th>
<th>Buffer Additions</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene Glycol (PEG) 100 ml</td>
<td>EDTA (100 mM)</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Dithiothreitol (DTT)</td>
<td>120 mg</td>
<td>Divalent Calcium Solution 0.5 ml</td>
<td>1 ml</td>
</tr>
<tr>
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<td>120 mg</td>
<td>Divalent Calcium Solution 0.5 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Oxidized Glutathione (GSSG)</td>
<td>150 mg</td>
<td>Reduced Glutathione (GSH)</td>
<td>150 mg</td>
</tr>
</tbody>
</table>

Ordering Information

**Product # Description**

- **43604 Pro-Matrix® Protein Refolding Buffer Kit**
  - Contains sufficient components to conduct 100 refolding experiments (1 ml each).
  - Includes: Base Refolding Buffers 1-9 (100 ml).
  - Includes: Base Refolding Buffers 1-9 (100 ml).
  - Includes: Base Refolding Buffers 1-9 (100 ml).
  - **$675**

---

**Table 13: Buffer additives.**

<table>
<thead>
<tr>
<th>Buffer Additions</th>
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<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Divalent Calcium Solution 0.5 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Oxidized Glutathione (GSSG)</td>
<td>150 mg</td>
<td>Reduced Glutathione (GSH)</td>
<td>150 mg</td>
</tr>
</tbody>
</table>

Ordering Information

**Product # Description**

- **89867 Pro-Matrix® Protein Refolding Kit**
  - Contains sufficient components to conduct 100 refolding experiments (1 ml each).
  - Includes: Base Refolding Buffers 1-9 (100 ml).
  - Includes: Base Refolding Buffers 1-9 (100 ml).
  - Includes: Base Refolding Buffers 1-9 (100 ml).
  - **$675**

---

**Figure 31. Set-up and handling of the Pro-Matrix® Protein Refolding Kit.**

Dispense 500 µl of the Base Refolding Buffers in nine tubes.

Add supplied additives as required for target protein to the above nine tubes. Adjust volumes to 500 µl.

Incorporate overnight. Analyze by electrophoresis.

---

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007
Protein Refolding

The following protocol is a generally applicable method useful in components, such as a reducing agent and a chelating agent, may be effective means for solubilizing aggregated proteins. Additional Inclusion Body Solubilization Reagent is designed to retrieve expressed aggregates are formed, it is very difficult to solubilize them. Pierce Inclusion Body Solubilization Reagent is效力retired expressed protein in soluble form after lysis and extraction procedures.

A proprietary denaturant contained in this reagent provides the greatest effectiveness for solubilizing aggregated proteins. Additional components, such as a reducing agent and a chelating agent, may be added to the reagent, depending on the particular application. The following protocol is a generally applicable method useful for moving protein from the insoluble inclusion body state into solution in a gentle, phased approach. This is the first and essential step before proceeding with insoluble protein refolding procedures. (See ProMatix™ Protein Refolding Kit, page 33.)

Inclusion Body purification. Recombinant proteins expressed in bacteria often form inclusion bodies, especially when they are expressed at high levels. It is not known exactly how they are formed, but it is thought that the protein within the inclusion body is partially or incompletely folded. The advantage of inclusion bodies is that they generally allow greater levels of expression, and they can be easily separated from a large proportion of bacterial cytoplasmic protein. The denaturant can be removed by dialysis before performing other protein assays.

Protein Refolding Using Dialysis Method

Materials required:
- B-PER® Bacterial Protein Extraction Reagent
- Inclusion Body Solubilization Reagent: For inclusion body purification
- DTT: Reducing agent
- Urea: Mild denaturant
- Slide-A-Lyzer® Dialysis Cassette: Dialysis (Product # 66410)

Protein Refolding

Because this reagent contains denaturant, the protein is denatured after solubilization. To obtain active protein, it is necessary to perform protein refolding. Several methods have been published describing protein refolding - a suggested protocol is provided for proceeding with refolding procedures. Because every protein possesses unique folding properties, the optimal refolding protocol for any given protein must be empirically determined.

Compatibility. The denaturant included in the Inclusion Body Solubilization Reagent will precipitate in SDS-PAGE sample buffer. However, this reagent is compatible with Pierce Coomassie Plus – The Better Bradford® Protein Assay Reagent (Product # 23236). The denaturant can be removed by dialysis before performing SDS-PAGE analysis and other protein assays.

Example Solubilization Protocol

1. Purify the inclusion body using B-PER® Bacterial Protein Extraction Reagent (Product # 78248). Be sure to follow the manufacturer-supplied instructions. Inclusion bodies purified by other methods are also applicable to the following protocol. The purity of the inclusion body may be analyzed by SDS-PAGE before performing the solubilization procedures. The purity of the inclusion body preparation will not affect the solubilization efficiency. However, if a sufficient amount of refolding reagent is desired, a greater than 90% purity is preferred.

2. Purify the inclusion body from bacteria using B-PER® Bacterial Protein Extraction Reagent and solubilize inclusion body protein using Inclusion Body Solubilization Reagent following respective protocols. If disulfide bonds are involved in the refolding, add DTT to 5 mM to the Inclusion Body Solubilization Reagent during the solubilization step.

3. Prepare 10 ml of Urea solution in a 25 ml glass or plastic beaker. Add 200 µl of Urea solution to 2 ml of inclusion body suspension is homogeneous. Shake the suspension for 30 minutes.

4. Add 0.01 ml of DTT to 5 µM to the Inclusion Body Solubilization Reagent by dialysis.

5. Collect the supernatant that contains the solubilized protein from the inclusion body. If a protein assay is desired, Pierce Coomassie Plus – The Better Bradford® Protein Assay Reagent (Product # 23236) is compatible. For SDS-PAGE analysis, it is necessary to remove the denaturant contained in the solubilization reagent by dialysis.

Protein Refolding Using Dialysis Method

Materials required:
- B-PER® Bacterial Protein Extraction Reagent: For inclusion body purification
- Inclusion Body Solubilization Reagent: For inclusion body solubilization
- DTT: Reducing agent
- Urea: Mild denaturant
- Slide-A-Lyzer® Dialysis Cassette: Dialysis (Product # 66410)

Suggested Refolding Protocol

1. Purify inclusion body from bacteria using B-PER® Bacterial Protein Extraction Reagent and solubilize inclusion body protein using Inclusion Body Solubilization Reagent following respective protocols. If disulfide bonds are involved in the refolding, add DTT to 5 mM to the Inclusion Body Solubilization Reagent during the solubilization step.

2. Prepare 10 ml of Urea solution in a 25 ml glass or plastic beaker. Add 200 µl of Urea solution to 2 ml of inclusion body suspension is homogeneous. Shake the suspension for 30 minutes.

3. Add 0.01 ml of DTT to 5 µM to the Inclusion Body Solubilization Reagent by dialysis.

4. Collect the supernatant that contains the solubilized protein from the inclusion body. If a protein assay is desired, Pierce Coomassie Plus – The Better Bradford® Protein Assay Reagent (Product # 23236) is compatible. For SDS-PAGE analysis, it is necessary to remove the denaturant contained in the solubilization reagent by dialysis.

5. Purify the inclusion body from bacteria using B-PER® Bacterial Protein Extraction Reagent and solubilize inclusion body protein using Inclusion Body Solubilization Reagent following respective protocols. If disulfide bonds are involved in the refolding, add DTT to 5 mM to the Inclusion Body Solubilization Reagent during the solubilization step.

6. Prepare 10 ml of Urea solution in a 25 ml glass or plastic beaker.

7. Add 0.01 ml of DTT to 5 µM to the Inclusion Body Solubilization Reagent by dialysis.

8. Collect the supernatant that contains the solubilized protein from the inclusion body. If a protein assay is desired, Pierce Coomassie Plus – The Better Bradford® Protein Assay Reagent (Product # 23236) is compatible. For SDS-PAGE analysis, it is necessary to remove the denaturant contained in the solubilization reagent by dialysis.

The easy-to-use Zea® Spin-Column Format dramatically improves results over standard drip-column methodologies, eliminating the need to wait for samples to emerge by gravity flow and the need to monitor fractions for protein recovery. Zea® Dosing Columns require no chromatographic system, cumbersome column preparation or equilibration and they can process multiple samples in ~8 minutes.

Zeba® Desalt Spin Columns

Zeba® Desalt Spin Columns contain a proprietary high-performance desalting resin, exclusive to Pierce, that offers exceptional desalting and protein-recovery characteristics compared to other commercially available resins (Figure 41). Samples containing as low as 25 µg/ml of protein can be processed, providing exceptional protein recovery and ≥95% retention of salts and other small molecules (<1,000 MW).

Highlight:
- Exceptional protein recovery
- Wide product offering accommodates your sample needs
- Easy-to-use with no cumbersome column preparation or equilibration
- No screening fractions for protein or waiting for protein to emerge by gravity flow
- Minimal sample dilution

Although numerous techniques and resins for desalting are available, most have many drawbacks, including significant sample loss, long processing times and the need to collect multiple fractions. Zea® Desalt Spin Columns provide excellent protein recovery without the limitations associated with other desalting methods.

With the introduction of Zea® Desalt Spin Columns in 2, 5 and 10 ml formats to complement the Micro and 0.5 ml versions, the Zea® Desalt Spin Column family of products allows processing of samples volumes ranging from 2 µl to 4 ml (Table 13).

Table 13. Recommended sample volumes for Zea® Spin Columns.

<table>
<thead>
<tr>
<th>Resin Size</th>
<th>Sample Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 µl micro-column</td>
<td>2-12 µl</td>
</tr>
<tr>
<td>5 µl column</td>
<td>200-700 µl</td>
</tr>
<tr>
<td>10 µl column</td>
<td>600-2,000 µl</td>
</tr>
</tbody>
</table>

The Zea® Desalt Spin Columns are available in different sizes to meet your specific requirements.

Zeba® Desalt Spin Columns

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007

References

For Technical or Customer Assistance, Call 800-874-3723 or Fax 800-842-5007 35www.piercenet.com/pop95d + E-mail Customer Service: CS@piercenet.com
**Protein Concentration**

ICON™ Concentrators are advanced disposable ultrafiltration centrifugal devices for concentration and diafiltration/buffer-exchange of biological samples such as enzymes, antigens or antibodies. The innovative conical design* and high-performance regenerated cellulose membrane provide excellent protein concentration and recovery from dilute protein samples. ICON™ Concentrators are available with MWCO of 9K and 20K in 7 and 20 ml volume sizes.

**Highlights:**
- Achieve 150- to 400-fold protein concentrations in less than 30 minutes
- Accommodate concentration volumes over a wide working range (7 ml = 1-7 ml and 20 ml = 5-20 ml ranges)
- Desalt and exchange buffers
- Uses the maximum membrane surface area, providing unsurpassed protein concentration
- Compatible with swinging-bucket or fixed-angle rotors
- No invert spin required
- Excellent recovery of dilute proteins

**Ordering Information**

<table>
<thead>
<tr>
<th>Product #</th>
<th>Description</th>
<th>Pkg.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>89884</td>
<td>ICON™ Concentrators, 7 ml, 9K MWCO</td>
<td>25/pk.</td>
<td></td>
</tr>
<tr>
<td>89885</td>
<td>ICON™ Concentrators, 20 ml, 9K MWCO</td>
<td>25/pk.</td>
<td></td>
</tr>
<tr>
<td>89886</td>
<td>ICON™ Concentrators, 7 ml, 20K MWCO</td>
<td>25/pk.</td>
<td></td>
</tr>
<tr>
<td>89887</td>
<td>ICON™ Concentrators, 20 ml, 20K MWCO</td>
<td>25/pk.</td>
<td></td>
</tr>
</tbody>
</table>

www.piercenet.com/icon

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**High-Performance Dialysis**

The new 30 ml (working range 12-30 ml) Slide-A-Lyzer™ Dialysis Cassette* was built for dialysis in a BIG way. It comes with its own internal buoy. The color-coded transparent frames of the Slide-A-Lyzer® Cassette Family allow you to instantly know the molecular weight cut-off of the membrane and view the placement of your needle during sample injection.

**Highlights:**
- The 30 ml cassette has the buoy built into its frame
- > 95% sample recovery
- No knots, caps, lids or clamps to loosen, fall off, open or leak
- A rigid frame that permits smooth and complete withdrawal of samples
- Color-coded frames indicate membrane MWCO

To view demos, see a complete list of products or to download an instruction booklet, visit www.piercenet.com/dialysis.
A comprehensive Refolding Guide is included in your Pro-Matrix™ Protein Refolding Kit. It includes details on isolating, solubilizing and purifying inclusion bodies; optimizing refolding conditions; and analyzing refolding yields.

• **High-yield refolding conditions** – reagents and conditions limited to those proven effective for high-concentration protein refolding experiments

• **Adjustable three-level matrix format** – customizes refolding experiments to specific protein, reduces optimization time and simplifies data interpretation

• **High-purity reagents** – offers quality reagents formulated using stringent standards so that consistent results are attained

**Product #** | **Description**
--- | ---
89867 | Pro-Matrix™ Protein Refolding Kit

**Bring your protein back into the fold.**

Restore native protein function with the Pro-Matrix™ Protein Refolding Kit.

The Pro-Matrix™ Protein Refolding Kit is an enabling tool for determining and optimizing conditions for restoring native protein function from inclusion bodies.