

PhosphoSafe[™] Extraction Reagent

Description

| | | |
|--------------------------------|-----------|---------|
| PhosphoSafe Extraction Reagent | 25 ml | 71296-3 |
| | 5 × 25 ml | 71296-4 |

PhosphoSafe Extraction Reagent efficiently extracts soluble proteins from mammalian and insect cell while preserving their phosphorylation state. This reagent contains the same formula as CytoBuster[™] Protein Extraction Reagent, but also includes four phosphatase inhibitors: sodium fluoride, sodium vanadate, β -glycerophosphate, and sodium pyrophosphate. PhosphoSafe is compatible with kinase assays, protein interaction analysis, and other applications. The reagent is compatible with protease inhibitors, kinase inhibitors, other phosphatase inhibitors, and with BCA Protein Assay Kit (Cat. No. 71285-3).

Storage

Store PhosphoSafe Extraction Reagent at -20°C if using within a few weeks. For storage longer than a few weeks, store PhosphoSafe at -70°C .

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Using PhosphoSafe™ Extraction Reagent

General considerations

Allow PhosphoSafe to thaw and gently swirl to mix prior to use. Depending on your experimental needs, the reagent should be dispensed into appropriate aliquots and stored at -20°C or -70°C . Repeated freeze/thaw cycles may lead to decreased activity. Extraction can be performed at room temperature. To minimize proteolysis, extraction can be performed on ice and protease inhibitors can be added (i.e., Cat. Nos. 539132 or 539134).

PhosphoSafe Extraction Reagent does not contain metal ions or metal chelators, but is compatible with the addition of these components. If precipitation occurs when adding metal ions, dilute the extracts with 1X PBS or reduce the amount of metal ion added to the reagent.

The following table gives the recommended volumes of PhosphoSafe Extraction Reagent to use for extraction of mammalian and insect cells grown in a monolayer or in suspension.

| Culture Format | Surface Area (cm^2) | Volume of PhosphoSafe |
|------------------|--------------------------------|-----------------------|
| 96-well Plate | 0.3 | 30 μl |
| 48-well Plate | 0.8 | 50 μl |
| 24-well Plate | 2.0 | 100 μl |
| 12-well Plate | 4.0 | 200 μl |
| 6-well Plate | 9.6 | 300 μl |
| 35-mm Dish | 9.6 | 300 μl |
| 60-mm Dish | 21.0 | 500 μl |
| 100-mm Dish | 55.0 | 1.0 ml |
| T-25 Flask | 25.0 | 500 μl |
| T-75 Flask | 75.0 | 1.5 ml |
| Suspension cells | 10^6 cells* | 150 μl |

*Suspension cells vary greatly in size; thus adjustment may be necessary.

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Extraction of monolayer cells

1. Aspirate culture medium from cells.
Optional: If components of the culture medium (i.e., phenol red) are inhibitory to protein analysis, rinse cells once with PBS (137 mM NaCl, 10 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4) or Hanks' Buffered Salts Solution (HBSS).
2. Add the recommended amount of PhosphoSafe™ Extraction Reagent (see table on page 1) and incubate at room temperature for 5 min.
3. To maximize recovery, scrape cell debris using a cell scraper (rubber policeman). Orient the plate so that all debris is pooled in the PhosphoSafe.
4. Transfer extract to a suitably size tube and spin for 5 min at 16,000 × g at 4°C.
5. Transfer supernatant (cell extract) to a new tube and proceed with analysis.

Note: Extracts prepared with PhosphoSafe can be used immediately or frozen at –20°C or –70°C until needed. Store extracts at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

Extraction of suspension cells

1. Collect cells by low speed centrifugation (e.g., 5 min at 2500 × g).
Optional: If components of the culture medium (i.e., phenol red) are inhibitory to reporter enzyme analysis, wash cells once with PBS or HBSS prior to PhosphoSafe addition. Collect cells by low speed centrifugation cells as above and discard supernatant. Drain the cell pellet well.
2. Resuspend the cells in PhosphoSafe Extraction Reagent using 150 µl per 10⁶ cells (optimal amount of PhosphoSafe may vary based on cell size).
3. Incubate at room temperature for 5 min.
4. Transfer the solution to a suitably sized tube and spin for 5 min at 16,000 × g at 4°C.
5. Transfer supernatant (cell extract) to a fresh tube and proceed with analysis.

Note: Extracts prepared with PhosphoSafe can be used immediately or frozen at –20°C or –70°C until needed. Store extracts at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

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