Cytobuster™ Protein Extraction Reagent

About the Kits

Cytobuster Protein Extraction Reagent	50 ml	71009-3
	250 ml	71009-4

Description

CytoBuster Protein Extraction Reagent is a proprietary formulation of detergents optimized for efficient extraction of soluble proteins from mammalian and insect cells. The unique composition of CytoBuster enables isolation of functionally active proteins without mechanical treatment such as sonication or freeze/thaw. CytoBuster Protein Extraction Reagent has been specifically formulated for use with Western blotting protocols, immunoprecipitation, and kinase/phosphatase assays. The reagent is compatible with protease inhibitors, kinase and phosphatase inhibitors, and with BCA protein assays. The clarified extract is compatible with the affinity supports offered by Novagen, including GST•Bind™, GST•Mag™, His•Bind®, His•Mag™, Strep-Tactin™, and S•Protein Agarose purification resin.

Storage

Store CytoBuster Protein Extraction Reagent at room temperature.

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A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
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Using CytoBuster™ Protein Extraction Reagent

General considerations

CytoBuster™ can be used directly for protein extraction at room temperature. If proteolysis must be minimized, CytoBuster may be chilled to 4°C and the extraction performed on ice, and a protease inhibitor cocktail (e.g. Cat. Nos. 539132 or 539134) may be added.

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

Culture Format	Surface Area (cm^2)	Volume of Cytobuster
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.

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 mMNa_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4) or Hanks' Buffered Salts Solution (HBSS).
2. Add the recommended amount of CytoBuster Protein Extraction Reagent (see above) 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 CytoBuster Protein Extraction Reagent.
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 CytoBuster Protein Extraction Reagent 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. Pellet the 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 adding CytoBuster Protein Extraction Reagent. Pellet cells as above and discard supernatant. Drain the cell pellet well.
2. Resuspend the cells in CytoBuster Protein Extraction Reagent using 150 µl per 10^6 cells (optimal amount of CytoBuster Protein Extraction Reagent may vary based on cell size).
3. Incubate at room temperature for 5 min.
4. Transfer to a suitable tube and spin for 5 min at 16,000 × g at 4°C.
5. Transfer cleared supernatant (cell extract) to a fresh tube and proceed with analysis.

Note: *Extracts prepared with CytoBuster 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.*