DUALxtract

Mammalian cell total protein extraction kit

User Manual

P07113S

Product

Kit for optimal extraction of total proteins from mammalian cells and tissues.

Contents

- Mammalian Cells Lysis Reagent (5 ml)

Storage

The reagent should be stored at 4°C.

Background

DUALXtract Mammalian Cell Total Protein Extraction Reagent is a ready-to-use solution designed for highly effective and convenient total protein extraction from any mammalian cultured cells or tissue samples at room temperature. The procedure is fast and simple: no sonication and no freeze-thaw cycles are required. Proteins extracted using this reagent are non-denatured, functionally active and therefore ideal for direct use in many common downstream applications, including 1D and 2D electrophoresis, Western blotting, electrophoretic mobility shift assay (EMSA), immunoprecipitation, affinity purification, enzymatic activity and reporter gene assays. Isolated proteins are compatible with quantification assays such as Bradford, Lowry and the BCA assay. The reagent provided is sufficient for:

- 2 extractions from 100 µl of wet cell pellet or
- 10 extractions from 100 mg of tissue.

Important notes

Protease inhibitor cocktail may be added to the DUALXtract Mammalian Cell Total Protein Extraction Reagent to minimize proteolysis.

The volume of the DUALXtract Mammalian Cell Total Protein Extraction Reagent used depends on the amount of cells and on the expected final protein concentration in the extract. The following recommendations can be considered for general guidance:

- Suspension cells: 20 volumes of lysis reagent to 1 volume of packed cells (e.g. 200 µl for $5x10^6$ cells)
- Adherent cells: 400 µl for 35 mm plate or 1000 µl for 100 mm plate
- Tissue: 500 µl/100 mg

Protocols

For adherent cells

- Remove the growth medium from the cells.

- Rinse cells once with PBS to remove residual medium. Discard washing buffer

- Cell lysis:

  For direct lysis in culture plates

  - Add an appropriate volume of DUALXtract Mammalian Cell Total Protein Extraction Reagent (400 µl for 35 mm plate or 1000 µl for 100 mm plate).
  - Incubate for 10 minutes at room temperature on a shaker.
  - Collect lysate from plates into a microcentrifuge tube (to maximize recovery, scrape cell debris using cell scraper).

  For lysis in a microcentrifuge tubes (generates protein extracts at higher concentration)

  - Collect cells by scraping in appropriate volume of PBS or by trypsinization.
  - Transfer cells to a microcentrifuge tube.
  - Pellet cells by centrifugation at 500 x g for 7 minutes and discard supernatant. Estimate the packed cell volume.
  - Add 20 volumes of DUALXtract Mammalian Cell Total Protein Extraction Reagent to 1 volume of packed cells. Resuspend cell pellet by vortexing.
  - Incubate for 10 minutes at room temperature on a shaker (900-1200 rpm).

- Clarify the lysate by centrifugation at 16,000-20,000 x g for 15 minutes.

- Transfer the supernatant to a new tube. Use the cell lysate immediately or store at -70°C for future use.

For suspension cultured cells

- Collect cells in an appropriate centrifuge tube. Pellet cells by centrifugation at 500 x g for 7 minutes and discard the supernatant.

- Rinse cells once with PBS to remove residual medium and repeat centrifugation step. Estimate the packed cell volume.

- Add 20 volumes of DUALXtract Mammalian Cell Total Protein Extraction Reagent to 1 volume of packed cells. Resuspend cell pellet by vortexing.

- Incubate for 10 minutes at room temperature on a shaker (900-1200 rpm).

- Clarify the lysate by centrifugation at 16,000-20,000 x g for 15 minutes.

- Transfer the supernatant to a new tube. Use the cell lysate immediately or store at -70°C for future use.

For tissue cells

- Weigh out the tissue sample. Transfer (either fresh or frozen) to a mortar and crush the tissue in liquid nitrogen with a pestle. During homogenization keep the tissue completely frozen to preserve functional and structural integrity of proteins.
  Note: complete tissue homogenization is a critical step for total protein yield.

- Transfer the tissue powder to a microcentrifuge tube and add an appropriate volume of DUALXtract Mammalian Cell Total Protein Extraction Reagent (500 µl for 100 mg of tissue).

- Resuspend tissue powder by vortexing and incubate for 10 minutes at room temperature on a shaker (900-1200 rpm).

- Clarify the lysate by centrifugation at 16,000-20,000 x g for 15 minutes.

- Transfer the supernatant to a new tube. Use the cell lysate immediately or store at -70°C for future use.

Troubleshooting

Low protein yield

Insufficient volume of cell lysis reagent used.
Add more cell lysis reagent.

Insufficient dispersion of cells.
Thoroughly vortex pelleted suspension cells following centrifugation. Adherent cells should be removed from the culture dish with a cell scraper or by trypsin treatment. Use non-confluent cells. Homogenize tissues thoroughly.

Insufficient cell lysis.
Prolong incubation with cell lysis reagent and shake more vigorously during incubation.

Cytoplasmic protein is bound to membrane proteins and is extracted with membrane protein fraction.
Check the properties of the contaminating cytoplasmic protein.

Low protein conc.

Excessive volume of lysis reagent used.
Decrease the volume of lysis reagent or increase number of cells

Protein activity is absent or low

Proteins are degraded.
Limit procedure time to a minimum and freeze samples immediately after extraction. Use protease inhibitor cocktail.

Related products

P07002	DUALXtract buffer set (standard size)
P07114	DUALXtract Cytoplasmic and Nuclear Protein Extraction Kit
P07501	DUALrefold membrane protein refolding kit

Support

Please see www.dualsystems.com for support and protocols. Please direct support inquiries to support@dualsystems.com or call +41 44 738 50 00.

Research use

This product is intended for research use only, not for diagnostic or therapeutic uses.

MSDS

Please see the accompanying MSDS for safety and handling instructions. Observe good laboratory practice guidelines and wear gloves, laboratory coat and glasses when handling the product.

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