DUALxtract

Nuclear and cytoplasmic protein extraction kit

User Manual

P07114S

Product

Kit for stepwise isolation of cytoplasmic proteins and nuclei/nuclear proteins from mammalian cells and tissue.

Contents

- Cell Lysis Buffer (2.5 ml)
- Nuclei Washing Buffer (7.5 ml)
- Nuclei Lysis Reagent (0.8 ml)

Storage

All components of the kit should be stored at 4°C.

Reagents to be supplied by user

- 1M DTT stock solution
- Protease inhibitor cocktail

Background

The DUALXtract Cytoplasmic and Nuclear Protein Extraction Kit is designed for rapid stepwise isolation of cytoplasmic proteins and nuclei or nuclear proteins from mammalian cultured cells and tissues. The extracted non-denatured cytoplasmic proteins are suitable for many different applications, including Western blotting, immunoassays, protein activity and reporter gene assays. Isolated cytoplasmic proteins can also be used in 1D and 2D electrophoretic analysis. Isolated intact nuclei are lysed with the nuclei lysis. Extracted nuclei can be used for many cell biology applications: isolation of chromatin, histones or nuclear RNA, isolation of total nuclear proteins for use in DNA-protein interaction assays (EMSA), 2D fractionation or other applications. Both, cytoplasmic and nuclear proteins, are compatible with protein quantification assays such as Bradford, Lowry and the BCA assay. The kit provides reagents sufficient for extraction of cytoplasmic and nuclear fractions from 5 samples of 50 µl wet cell pellet or 100 mg of tissue.

Important notes

- All extractions should be performed on ice.

- The volume of the cell lysis buffer used depends on the amount of cells and on the expected final protein concentration in the extract. The following recommendations can be considered as a general guidance:

  - use 10 volumes of cell lysis buffer over packed cell volume, e.g. 200 µl for $5x10^6$ cells.
  - use 500 µl of cell lysis buffer for 100 mg of tissue

- DTT is required but not supplied with the kit. Always prepare 0.1 M DTT freshly from a 1 M stock. Store 1 M DTT stocks at -20°C, thaw on ice and place back at -20°C immediately after use. Discard after 10 freeze/thaw cycles. Prepare 0.1 M DTT by adding 5 µl of 1 M DTT stock to 45 µl of deionized water.

- Add 0.1 M DTT to the provided reagents immediately before use:
  Add 2 µl DTT (0.1M) to Cell Lysis Buffer (200 µl final volume)
  Add 30 µl DTT (0.1M) to Nuclei Washing Buffer(1000 µl final volume)

- Protease inhibitor cocktail may be added to cell lysis buffer and nuclei washing buffer to minimize proteolysis.

- Protein extracts can be directly used for gel electrophoresis.
  Precipitation and buffer change are not required.

Protocols

Suspension culture cells

Cell harvesting and lysis

- Pellet cells by centrifugation at 250 x g for 5 min. Discard the supernatant.

- Resuspend cells in PBS. Repeat centrifugation step. Discard the supernatant. Estimate the packed cell volume.

- Add 10 volumes of Cell lysis buffer (containing appropriate protease inhibitors and 1 mM DTT) to 1 volume of packed cells.

- Vortex for 10 s, set on ice for 10 min and vortex again.

Adherent cells

Cell harvesting and lysis

- Remove the growth medium from the cells to be assayed. Rinse the cells once with PBS.
- Collect cells in PBS using a cell scraper or by trypsinization.
- Transfer the cells to a microcentrifuge tube.
- Pellet cells by centrifugation at 250 x g for 5 min and discard supernatant. Estimate the packed cell volume.
- Add 10 volumes of Cell lysis buffer (containing appropriate protease inhibitors and 1 mM DTT) to 1 volume of packed cells.
- Vortex for 10 s, set on ice for 10 min and vortex again.

Extraction of cytoplasmic proteins

- Separate the cytoplasmic fraction from nuclei by centrifugation at 500 x g for 7 minutes at 4°C. Carefully remove the supernatant (cytoplasmic protein extract) to a new tube.
- Set the nuclei pellet on ice.

  Clear cytoplasmic protein extract by centrifugation at 20,000 x g for 15 min at 4°C, transfer the supernatant to a new tube. Use directly or store at -70°C for later analysis.

Washing of nuclei

- Wash nuclei twice:
  Add 500 µl of the Nuclei washing buffer (containing appropriate protease inhibitors and 3 mM DTT) to nuclei pellet, vortex briefly and set on ice for 2 min. Centrifuge at 500 x g for 7 minutes at 4°C and carefully remove supernatant.
  Note: The purity of the extracted nuclei can be assessed with a fluorescent microscope. Prepare a staining mixture of 0.01% acridine orange and 0.01% etidium bromide (1:1 v/v). Add 3 µl of the staining mixture per 50 µl of nuclei suspension. Nuclei will stain blue, whereas cytoplasmic contamination, if present, will stain light blue.

Lysis of nuclei

- Add 150 µl of the Nuclei lysis reagent to the nuclei pellet. Vortex briefly and shake for 15 min at 4°C (900-1200 rpm).

- Clear nuclear lysate by centrifugation at 20,000 x g for 5 min at 4°C. Transfer the supernatant (nuclear protein extract) to a new tube. Use immediately or store at -70°C for later analysis.

Tissue

Tissue preparation and cell lysis

- Rinse the fresh tissue sample briefly with ice cold PBS and blot dry. Keep the tissue sample on ice in a suitable container.

- Homogenize the tissue gently in PBS (with protease inhibitors).

- Remove non-homogenized tissue by filtration through gauze filter.

- Transfer homogenate into a microcentrifuge tube and spin down by centrifugation at 250 x g for 5 min at 4°C. Remove supernatant.

- Estimate the packed cell volume.

  Add 10 volumes of Cell lysis buffer (containing appropriate protease inhibitors and 1 mM DTT) to 1 volume of packed cells.

- Vortex briefly and set on ice for 10 min.

Extraction of cytoplasmic proteins

- Separate the cytoplasmic fraction from nuclei by centrifugation at 500 x g for 7 minutes at 4°C. Carefully remove the supernatant (cytoplasmic protein extract) to a new tube.

- Set the nuclei pellet on ice.

  Clear cytoplasmic protein extract by centrifugation at 20,000 x g for 15 min at 4°C, transfer the supernatant to a new tube. Use directly or store at -70°C for later analysis.

Washing of nuclei

- Add 500 µl of the Nuclei washing buffer (containing appropriate protease inhibitors and 1 mM DTT) to nuclei pellet, vortex briefly and set on ice for 2 min. Centrifuge at 500 x g for 7 min at 4°C and carefully remove supernatant. Repeat 1-2 times.

Note: The purity of the extracted nuclei can be assessed with a fluorescent microscope. Prepare a staining mixture of 0.01% acridine orange and 0.01% etidium bromide (1:1 v/v). Add 3 µl of the staining mixture per 50 µl of nuclei suspension. Nuclei will stain blue, whereas cytoplasmic contamination, if present, will stain light blue.

- Estimate the nuclei pellet volume.

Lysis of nuclei

- Add 10 volumes of the Nuclei lysis reagent to the nuclei pellet. Vortex briefly and shake for 15 min at 4°C (900-1200 rpm).

- Clear nuclear lysate by centrifugation at 20,000 x g for 5 min at 4°C. Transfer the supernatant (nuclear protein extract) to a new tube. Use immediately or store at -70°C for later analysis.

Troubleshooting

Low yield of cytoplasmic proteins

Suboptimal volume of cell lysis buffer used.
Use volumes appropriate for the sample type and number of cells. For recommendations please see p.2.

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 gently but thoroughly.

Low yield of nuclear proteins

Incomplete recovery of nuclei.
Prolong centrifugation in step 3 to collect all nuclei.

Clumping of nuclei.
Damaged nuclei release DNA, which can cause nuclei clumping and reduce yield. To avoid nuclei damage, perform the isolation rapidly at 4° C.

Low protein concentration

Insufficient number of cells per volume of lysis buffer.
Decrease the volume of lysis buffer or increase the number of cells.

Low protein activity

Samples not kept cold during the procedure.

Keep samples on ice, centrifuge at 4°C and freeze samples immediately after extraction. Minimize time of the procedure.

Proteases in the sample.

Use protease inhibitor cocktail.

Cross-contamination between nuclear and cytoplasmic fractions

Incomplete lysis of cells.

Increase vortexing time to completely resuspend the cell pellet.

Incomplete homogenization or overhomogenization of tissue.
Homogenization time and conditions should be optimized.

Related products

P07002	DUALXtract buffer set (standard size)
P07113	DUALXtract Mammalian Cell Lysis Reagent
P07115	DUALXtract Membrane 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.