

ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit

COMPONENTS OF THE KIT

Component	#K0311 (50 preps)
Cell Lysis Buffer	25 ml
Nuclei Washing Buffer	3 x 25 ml
Nuclei Storage Buffer	7.5 ml
Nuclei Lysis Reagent	0.8 ml
1M DTT	0.3 ml

STORAGE

All components of the kit except 1M DTT should be stored at 4°C. Aliquot 1M DTT and store at -20°C.

DESCRIPTION

The ProteoJET™ 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 can either be lysed with the nuclei lysis reagent or stored frozen in the provided nuclei storage buffer for further use.

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 50 samples of 50 µl wet cell pellet or 100 mg of tissue.

ADDITIONAL MATERIALS REQUIRED

- Protease inhibitor cocktail
- Microcentrifuge
- Microcentrifuge tubes
- Cell scraper
- Vortex mixer
- PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na₂HPO₄, 2 mM KH₂PO₄, pH 7.4)
- Shaker

IMPORTANT NOTES

- The extraction 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 5x10⁶ cells.
 - use 500 µl of cell lysis buffer for 100 mg of tissue.
- Dilute 1 M DTT to a final concentration of 0.1 M by adding 5 µl of 1 M DTT to 45 µl of deionized water. Store at -20°C.
- Add 0.1 M DTT to the provided reagents immediately before use:

Buffer	Required volume per prep.	0.1M DTT
Cell lysis buffer	200 µl	2 µl
Nuclei washing buffer	1000 µl	30 µl
Nuclei storage buffer	150 µl	7.5 µl

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

PROTOCOLS

Protocol for cultured cells

Step	Procedure
1. Cell harvesting and lysis	<p>a) for suspension cells</p> <ul style="list-style-type: none"> • 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 (with protease inhibitors and DTT) to 1 volume of packed cells. • Vortex for 10 s, set on ice for 10 min and vortex again.
	<p>b) for adherent cells</p> <ul style="list-style-type: none"> • 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 (with protease inhibitors and DTT) to 1 volume of packed cells. • Vortex for 10 s, set on ice for 10 min and vortex again.

Step	Procedure
2. Extraction of cytoplasmic proteins	<ul style="list-style-type: none"> • Separate the cytoplasmic fraction from nuclei by centrifugation at 500 x g for 7 minutes at 4°C. Carefully remove the supernatant (<u>cytoplasmic protein extract</u>) 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.
3. Washing of nuclei	<ul style="list-style-type: none"> • Wash nuclei twice: Add 500 µl of the Nuclei washing buffer (with protease inhibitors and 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. <i>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.</i> • Add 150 µl of ice cold Nuclei storage buffer (with protease inhibitors and DTT) to the nuclei pellet. Pipette up and down 5-10 times to break up any clumps. Proceed with lysis or store frozen at -70°C.
4. Lysis of nuclei	<ul style="list-style-type: none"> • Add 1/10 volume of the Nuclei lysis reagent to nuclei suspension. 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 (<u>nuclear protein extract</u>) to a new tube. Use immediately or store at -70°C for later analysis.

Protocol for tissues

Step	Procedure
1. Preparation of tissue and cell lysis	<ul style="list-style-type: none"> 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 (with protease inhibitors and DTT) to 1 volume of packed cells. Vortex briefly and set on ice for 10 min.
2. Extraction of cytoplasmic proteins	<ul style="list-style-type: none"> Separate the cytoplasmic fraction from nuclei by centrifugation at 500 x g for 7 min at 4°C. Carefully transfer the supernatant (<u>cytoplasmic protein extract</u>) to a new tube. Set the nuclei pellet on ice. Clear cytoplasmic protein extract by centrifugation at 20,000 x g for 15 min, transfer to a new tube. Use directly or store at -70°C.

Step	Procedure
3. Washing of nuclei	<ul style="list-style-type: none"> Add 500 µl of the Nuclei washing buffer (with protease inhibitors and 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. <i>Note.</i> 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. Add 10 volumes of ice cold Nuclei storage buffer (with protease inhibitors and DTT) to 1 volume of nuclei pellet. Pipette up and down 5-10 times to break up any clumps. Proceed with lysis or store frozen at -70°C.
4. Lysis of nuclei	<ul style="list-style-type: none"> Add 1/10 volume of the Nuclei lysis reagent. 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 (<u>nuclear protein extract</u>) to a new tube. Use immediately or store at -70°C for later analysis.

QUALITY CONTROL

The ProteoJET™ Cytoplasmic and Nuclear Protein Extraction Kit is tested using the extraction protocol with suspension cells. Extracted protein fractions are separated in 12% SDS-PAGE, transferred to PVDF membrane and blotted for the presence of tubulin and histone H4. The intact nuclei are assayed with the fluorescent microscope.

TROUBLESHOOTING

Problem	Cause & Solution
Low yield of cytoplasmic proteins	<p>Suboptimal volume of cell lysis buffer used. Use volumes appropriate for the sample type and number of cells. For recommendations please see p.2.</p> <p>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.</p>
	<p>Incomplete recovery of nuclei. Prolong centrifugation in step 3 to collect all nuclei.</p> <p>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.</p>
Low yield of nuclear proteins	
Low protein concentration	<p>Insufficient number of cells per volume of lysis buffer. Decrease the volume of lysis buffer or increase the number of cells.</p>
Low protein activity	<p>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.</p> <p>Proteases in the sample. Use protease inhibitor cocktail.</p>
Cross-contamination between nuclear and cytoplasmic fractions	<p>Incomplete lysis of cells. Increase vortexing time to completely resuspend the cell pellet.</p>
	<p>Incomplete homogenization or overhomogenization of tissue. Homogenization time and conditions should be optimized.</p>

RELATED PRODUCTS

Product	Amount	Catalog #
Spectra™ Multicolor Broad Range Protein Ladder	2 x 250 µl	SM1841
PageRuler™ Unstained Protein Ladder	2 x 250 µl	SM0661
PageRuler™ Prestained Protein Ladder	2 x 250 µl	SM0671
PageRuler™ Plus Prestained Protein Ladder	2 x 250 µl	SM1811
Unstained Protein Molecular Weight Marker	2 x 1000 µl	SM0431
Prestained Protein Molecular Weight Marker	2 x 250 µl	SM0441
PageBlue™ Protein Staining Solution	1 liter	R0571
PageSilver™ Silver Staining Kit	25 preps	K0681
ProteoJET™ Mammalian Cell Lysis Reagent	250 ml	K0301
10X Tris-glycine-SDS Electrophoresis Buffer	1 liter	B46
10X Tris-glycine Electrophoresis Buffer	1 liter	B47
10X Tris-tricine-SDS Electrophoresis Buffer	1 liter	B48
DualColor™ Protein Loading Buffer Pack	1000 samples	R1011
Protein Loading Buffer Pack	2000 samples	R0891
Bradford Reagent, ready-to-use	1 liter	R1271
Bovine Serum Albumin Standard Set, ready-to-use	7 x 4 ml	R1281
Bovine Gamma Globulin Standard Set, ready-to-use	7 x 4 ml	R1291

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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