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ab221978
Nuclear Extract Kit

For the preparation of nuclear extracts from mammalian cells and tissue.

This product is for research use only and is not intended for diagnostic use.

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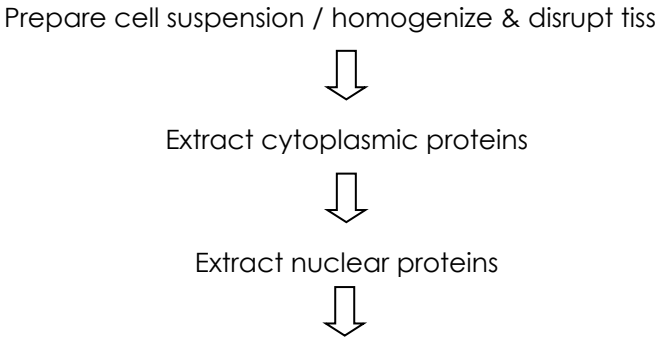
1. Overview

ab221978 provides the simple and selective method for extracting nuclear proteins used for a variety of applications. These applications may include western blotting, protein-DNA binding assays, nuclear enzyme assays, and the others requiring optimized nuclear proteins. The Nuclear Extraction Kits are also specifically designed to meet the requirements of nuclear extracts used in various assays. The Nuclear Extraction Kits can be used to extract nuclear proteins from mammalian cells and tissue samples. Total yield can be up to 100 µg per optimal extraction, although results may somewhat vary depending on the cell or tissue type.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.

- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit as given in the table upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
10X Nuclear Extraction Hypotonic Buffer	6 mL	4°C
1M Nuclear Extraction Dithiothreitol	60 µL	-20°C
100X Nuclear Extraction Protease Inhibitor Cocktail	650 µL	-20°C

10X Nuclear Extraction PBS	100 mL	4°C
50X Nuclear Extraction Phosphatase Inhibitors	2 x 5 mL	-20°C
2X Nuclear Extraction Buffer	3 mL	4°C
10% Nonidet P-40 Assay Reagent	6 mL	RT

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 15 mL and/or 50 mL conical tubes pre-cooled to 4°C.
- Centrifuge with swinging buckets adapted to 15 mL conical tubes pre-cooled to 4°C.
- Microfuge tubes (1.5 mL) pre-cooled to 4°C.
- Micro-centrifuge pre-cooled to 4°C.
- Platform rocker.
- Dounce homogenizer (for fresh tissue samples only).

8. Technical Hints

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

9.1 1X PBS/Phosphatase Inhibitor Solution

Determine the amount of PBS/Phosphatase Inhibitor Solution required using the table below. Adjust the volume according to the total number of cells being lysed. Keep on ice and use within the same day.

Reagent	60 mm plate ~3.5 x 106 cells	100 mm plate ~7 x 106 cells	150 mm plate ~1.5 x 107 cells
Nuclear Extraction PBS (10X)	0.6 mL	1 mL	1.5 mL
Distilled Water	5.28 mL	8.8 mL	13.2 mL
Nuclear Extraction Phosphatase Inhibitors	0.12 mL	0.2 mL	0.3 mL
Total Volume	6 mL	10 mL	15 mL

9.2 Complete Hypotonic Buffer (1X)

Determine the amount of Complete Hypotonic Buffer (1X) that will be required using the table below. Adjust the volume according to the total number of cells being lysed. Keep on ice and use within the same day.

Reagent	60 mm plate ~3.5 x 106 cells	100 mm plate ~7 x 106 cells	150 mm plate ~1.5 x 107 cells
Nuclear Extraction Hypotonic Buffer (10X)	25 µL	50 µL	100 µL
Nuclear Extraction Phosphatase Inhibitors (50X)	5 µL	10 µL	20 µL
Nuclear Extraction Protease Inhibitors (100X)	2.5 µL	5 µL	10 µL
Distilled Water	217.5 µL	435 µL	870 µL
Total Volume	250 µL	500 µL	1,000 µL

9.3 Nuclear Extraction Dithiothreitol

To prepare 10 mM dithiothreitol (DTT), dilute 1:100 in distilled water. The 1 M and 10 mM DTT solutions are stable for six months when stored at-20°C.

9.4 Complete Nuclear Extraction Buffer (1X)

Determine the amount of 1X Extraction Buffer that will be required using the table below. Adjust the volume according to the total number of cells being lysed. Keep on ice and use within the same day.

Reagent	60 mm plate ~3.5 x 106 cells	100 mm plate ~7 x 106 cells	150 mm plate ~1.5 x 107 cells
Nuclear Extraction Buffer (2X)	25 µL	50 µL	75 µL
Nuclear Extraction Protease Inhibitors (100X)	0.5 µL	1 µL	1.5 µL
Nuclear Extraction Phosphatase Inhibitors (50X)	1 µL	2 µL	3 µL
DTT (10 mM)	5 µL	10 µL	15 µL
Distilled Water	18.5 µL	37 µL	55.5 µL
Total Volume	50 µL	100 µL	150 µL

10. Assay Procedure

10.1 Purification of Cellular Nuclear Extracts:

The following procedure can be used for a 15 mL cell suspension grown in a T75 flask or for adherent cells grown in a 100 mm dish to 80-90% confluency, where 107 cells yields approximately 50 µg of nuclear protein. Adjust the volumes as needed according to the number of cells. Keep all solutions and extracted cytoplasmic and nuclear extracts on ice during the entire protocol.

10.1.1 Collect ~107 cells in pre-chilled 15 mL tubes. Collect adherent cells by scraping instead of using proteolytic enzymes.

10.1.2 Centrifuge suspended cells at 300 x g for five minutes at 4°C.

10.1.3 Discard the supernatant. Resuspend the cell pellet in 5 mL of ice-cold PBS/Phosphatase Inhibitor Solution and centrifuge at 300 x g for five minutes at 4°C. Repeat PBS/Phosphatase Inhibitor Solution wash one more time.

10.1.4 Discard the supernatant. Add 500 µL ice-cold 1X Complete Hypotonic Buffer. Mix gently by pipetting and transfer resuspended pellet to a pre-chilled 1.5 mL micro-centrifuge tube.

10.1.5 Incubate cells on ice for 15 minutes to allow cells to swell.

10.1.6 Add 100 µL of 10% NP-40 Assay Reagent (Item No. 600009). Mix gently by pipetting.

10.1.7 Centrifuge at 14,000 x g for 30 seconds at 4°C in a micro-centrifuge. Transfer the supernatant which contains the cytosolic fraction to a new tube and store at -80°C.

10.1.8 Resuspend the pellet in 100 µL ice-cold Complete Nuclear Extraction Buffer (1X). Vortex vigorously for 15 seconds then gently rock the tube on ice for 15 minutes. Vortex vigorously for 30 seconds and gently rock for an additional 15 minutes.

10.1.9 Centrifuge at 14,000 x g for 10 minutes at 4°C. The supernatant contains the nuclear fraction. Aliquot to clean chilled tubes, keeping a small aliquot aside for protein quantification, flash freeze and store at -80°C. Avoid freeze/thaw cycles. The extracts are ready to use.

10.1.10 Quantify the protein concentration using the BCA Protein Assay Kit (ab102536) or a similar protein quantification assay.

NOTE: The presence of some detergents may interfere with the protein quantification assay. Use the 1X Extraction Buffer as the blank and for diluting nuclear samples 1:10 and 1:50, and use the 1X Hypotonic Buffer as the blank and for diluting cytoplasmic samples.

10.2 Purification of Tissue Extracts:

NOTE: We suggest that only fresh tissue be used in the preparation outlined below.

10.2.1 Weigh a fresh tissue sample and cut into very small pieces using a clean razor blade. Collect the pieces in a pre-chilled, clean Dounce homogenizer.

10.2.2 While keeping the sample on ice, add 3 mL of ice-cold 1X Complete Hypotonic Buffer supplemented with DTT and NP-40 (3 µL of 1M DTT and 3 µL of 10% NP-40) per gram of tissue.

10.2.3 Homogenize the sample with a Dounce homogenizer or a polytron device and incubate on ice for 15 minutes.

10.2.4 Transfer to prechilled micro-centrifuge tubes and centrifuge at 300 x g for 10 minutes at 4°C and transfer the supernatant into a pre-chilled micro-centrifuge tube labeled "cytosolic fraction" (keep on ice).

10.2.5 Although the tissue is homogenized, most of the pelleted cells from step 4 are not yet lysed. Gently resuspend the cells in 500 µL of 1X Complete Hypotonic Buffer by pipetting up and down several times. Transfer to a pre-chilled micro-centrifuge tube.

10.2.6 Incubate cells on ice for an additional 15 minutes.

10.2.7 Add 50 µL of 10% NP-40. Mix by gently pipetting up and down.

10.2.8 Centrifuge at 14,000 x g for 30 seconds at 4°C in a micro-centrifuge. Transfer the supernatant to the cytosolic fraction tube from step T4 and store at -80°C.

10.2.9 Continue with Step 10.1.8.

11. Troubleshooting

Problem	Reason	Solution
Low protein concentration in cytoplasmic fraction	Incorrect volumes or mistake made in addition of buffers used for lysis or extraction	Make buffers carefully
	Volume of lysis or extraction buffer does not correspond to correct number of cells	Count cells and use appropriate buffer volumes
	Cell pellet not disrupted after the addition of hypotonic buffer	Gently pipette after adding Hypotonic Buffer so that the cell pellet is disrupted
	Incomplete lysis of cells	After adding 10% Nonidet P-40 check lysis using a microscope
Low protein concentration in nuclear fractions	Incorrect volumes or mistake made in addition of buffers used for lysis or extraction	Make buffers carefully

	Volume of Hypotonic Extraction Buffer does not correspond to correct number of cells	Count cells and use appropriate buffer volumes
	Cell pellet not disrupted after the addition of Hypotonic Buffer	Gently pipette after adding Hypotonic Buffer so that the cell pellet is disrupted
	Incomplete lysis of cells	After adding 10% Nonidet P-40 check lysis using a microscope
	Nuclear fraction lost in cytoplasmic fraction	Reduce the centrifuge time after adding 10% Nonidet P-40 (keep to under 30 seconds)
No or low protein yield in either cytoplasmic or nuclear fractions	If above causes have been corrected, cell type might not be compatible with this isolation procedure	Conditions of kit may need to be optimized for specific cell or tissue type
No or low amount of protein activity in applicable assay (i.e., transcription factor assay, western blot, EMSA, etc.)	Proteins in cytoplasmic and nuclear fractions may be degraded	Keep proteins at low temperatures during all steps of the procedure
		Limit time it takes to complete procedure
		Flash freeze aliquots of nuclear and cytoplasmic fractions immediately
		Avoid freeze/thaw cycles
		Check that protease inhibitors and phosphatase inhibitors have been added to buffers as outlined in kit booklet and quick guide

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)