Nuclear Extraction Kit

Item No. 10009277

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

The kit will arrive packaged as a -20°C. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
10009301	Nuclear Extraction Hypotonic Buffer (10X)	1 vial/6 ml	4°C
10009302	Nuclear Extraction Dithiothreitol (1 M)	1 vial/60 µl	-20°C
10009303	Nuclear Extraction Protease Inhibitor Cocktail (100X)	1 vial/650 µl	-20°C
10009304	Nuclear Extraction PBS (10X)	1 vial/100 ml	4°C
10009305	Nuclear Extraction Phosphatase Inhibitors (50X)	2 vials/5 ml	-20°C
10009306	Nuclear Extraction Buffer (2X)	1 vial/3 ml	4°C
600009	Nonidet P-40 Assay Reagent (10%)	1 vial/6 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

Kit components may be stored at -20°C prior to use. After use we recommend each kit component be stored according to the temperatures listed in the booklet.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. 15 ml and/or 50 ml conical tubes pre-cooled to 4°C
2. Centrifuge with swinging buckets adapted to 15 ml conical tubes pre-cooled to 4°C
3. Microfuge tubes (1.5 ml) pre-cooled to 4°C
4. Microcentrifuge pre-cooled to 4°C
5. Platform Rocker
6. Dounce homogenizer (for fresh tissue samples only)

INTRODUCTION

About This Assay

Preparation of nuclear extracts is the first step in examining transcription factor activity. Cayman's Nuclear Extraction Kit is formulated for the quick and simple isolation of nuclear and cytoplasmic fractions from cultured cells and tissue homogenates (see Figure 1 below). The proteins isolated using this kit can be used successfully in Cayman's Transcription Factor Assay Kits as well as standard electrophoretic mobility shift assays (EMSAs) and western blotting applications.

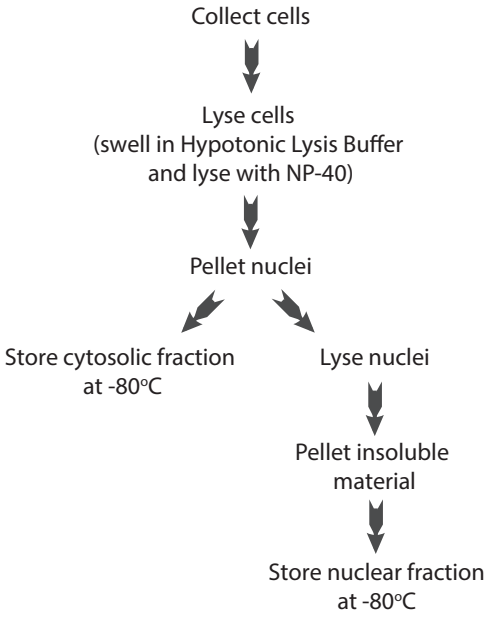

Figure 1. A flowchart summarizing the nuclear extraction protocol.

BUFFER PREPARATION

Reagent Preparation

1. **PBS/Phosphatase Inhibitor Solution (1X)**

   Determine the amount of PBS/Phosphatase Inhibitor Solution required using Table 1. 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 10^6 cells	100 mm plate ~7 x 10^6 cells	150 mm plate ~1.5 x 10^7 cells
Nuclear Extraction PBS (10X) (Item No. 10009304)	0.6 ml	1 ml	1.5 ml
Distilled Water	5.28 ml	8.8 ml	13.2 ml
Nuclear Extraction Phosphatase Inhibitors (50X) (Item No. 10009305)	0.12 ml	0.2 ml	0.3 ml
Total Volume	**6 ml**	**10 ml**	**15 ml**

Table 1. Preparation of PBS/Phosphatase Inhibitor Solution (1X)

2. Complete Hypotonic Buffer (1X)

Determine the amount of Complete Hypotonic Buffer (1X) that will be required using Table 2. 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 10^6 cells	100 mm plate ~7 x 10^6 cells	150 mm plate ~1.5 x 10^7 cells
Nuclear Extraction Hypotonic Buffer (10X) (Item No. 10009301)	25 µl	50 µl	100 µl
Nuclear Extraction Phosphatase Inhibitors (50X) (Item No. 10009305)	5 µl	10 µl	20 µl
Nuclear Extraction Protease Inhibitors (100X) (Item No. 10009303)	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**

Table 2. Preparation of Complete Hypotonic Buffer (1X)

3. Nuclear Extraction Dithiothreitol (Item No. 10009302)

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.

4. Complete Nuclear Extraction Buffer (1X)

Determine the amount of 1X Extraction Buffer that will be required using Table 3. 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 10^6 cells	100 mm plate ~7 x 10^6 cells	150 mm plate ~1.5 x 10^7 cells
Nuclear Extraction Buffer (2X) (Item No. 10009306)	25 µl	50 µl	75 µl
Nuclear Extraction Protease Inhibitors (100X) (Item No. 10009303)	0.5 µl	1 µl	1.5 µl
Nuclear Extraction Phosphatase Inhibitors (50X) (Item No. 10009305)	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**

Table 3. Preparation of Complete Nuclear Extraction Buffer (1X)

EXTRACTION PROTOCOL

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 10^7 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.

C1. Collect ~10^7 cells in pre-chilled 15 ml tubes. Collect adherent cells by scraping instead of using proteolytic enzymes.

C2. Centrifuge suspended cells at 300 x g for five minutes at 4°C.

C3. 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.

C4. 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 microcentrifuge tube.

C5. Incubate cells on ice for 15 minutes to allow cells to swell.

C6. Add 100 µl of 10% NP-40 Assay Reagent (Item No. 600009). Mix gently by pipetting.

C7. Centrifuge at 14,000 x g for 30 seconds at 4°C in a microcentrifuge. Transfer the supernatant which contains the cytosolic fraction to a new tube and store at -80°C.

C8. 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.

C9. 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.

C10. Quantify the protein concentration using Cayman's Protein Determination Kit (Item No. 704002) 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.

Purification of Tissue Extracts

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

T1. 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.

T2. 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.

T3. Homogenize the sample with a Dounce homogenizer or a polytron device and incubate on ice for 15 minutes.

T4. Transfer to prechilled microcentrifuge tubes and centrifuge at 300 x g for 10 minutes at 4°C and transfer the supernatant into a pre-chilled microcentrifuge tube labeled “cytosolic fraction” (keep on ice).

T5. 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 microcentrifuge tube.

T6. Incubate cells on ice for an additional 15 minutes.

T7. Add 50 µl of 10% NP-40. Mix by gently pipetting up and down.

T8. Centrifuge at 14,000 x g for 30 seconds at 4°C in a microcentrifuge. Transfer the supernatant to the cytosolic fraction tube from step T4 and store at -80°C.

T9. Continue with Step C8 in the Cellular Nuclear Extracts protocol.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low protein concentration in cytoplasmic fraction	A. Incorrect volumes or mistake made in addition of buffers used for lysis or extraction B. Volume of lysis or extraction buffer does not correspond to correct number of cells C. Cell pellet not disrupted after the addition of hypotonic buffer D. Incomplete lysis of cells	A. Make buffers carefully B. Count cells and use appropriate buffer volumes C. Gently pipette after adding Hypotonic Buffer so that the cell pellet is disrupted D. After adding 10% Nonidet P-40 check lysis using a microscope E. Check expiration date on reagents
Low protein concentration in nuclear fractions	A. Incorrect volumes or mistake made in addition of buffers used for lysis or extraction B. Volume of Hypotonic Extraction Buffer does not correspond to correct number of cells C. Cell pellet not disrupted after the addition of Hypotonic Buffer D. Incomplete lysis of cells E. Nuclear fraction lost in cytoplasmic fraction	A. Make buffers carefully B. Count cells and use appropriate buffer volumes C. Gently pipette after adding Hypotonic Buffer so that the cell pellet is disrupted D. After adding 10% Nonidet P-40 check lysis using a microscope E. Reduce the centrifuge time after adding 10% Nonidet P-40 (keep to under 30 seconds) F. Check expiration date on reagents

Problem (cont.)	Possible Causes (cont.)	Recommended Solutions (cont.)
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	A. Keep proteins at low temperatures during all steps of the procedure B. Limit time it takes to complete procedure C. Flash freeze aliquots of nuclear and cytoplasmic fractions immediately D. Avoid freeze/thaw cycles E. Check that protease inhibitors and phosphatase inhibitors have been added to buffers as outlined in kit booklet and quick guide

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.