Nuclear Extraction Kit

Item No. 10009277

Customer Service 800.364.9897 * **Technical Support** 888.526.5351

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GENERAL INFORMATION

Materials Supplied

The kit will arrive packaged as a -20°C kit and the inhibitors will arrive packaged at -80°C. For best results, store 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	-80°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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.**

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.

For research use only. Not for human or diagnostic use.

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. 5 and 10 ml pipettes
2. Adjustable pipettors
3. Cell scraper
4. 15 ml and/or 50 ml conical tubes pre-cooled to 4°C
5. Centrifuge with swinging buckets adapted to 15 ml conical tubes pre-cooled to 4°C
6. Microfuge tubes (1.5 ml) pre-cooled to 4°C
7. Microcentrifuge pre-cooled to 4°C
8. Platform Rocker
9. Distilled water
10. 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 that can be used successfully in Cayman's Transcription Factor Assay Kits. The proteins isolated using this kit can also be used in electrophoretic mobility shift assays (EMSA) and western blotting applications.

Each kit provides reagents for 50 extractions from ~1 x 10^7 cells, corresponding to adherent cells grown to 80-90% confluency in a 100 mm tissue culture dish or suspension cells. Cytoplasmic and nuclear extracts can also be prepared directly from tissue. The cells are first collected in ice-cold phosphate buffered saline (PBS) in the presence of phosphatase inhibitors. The phosphatase inhibitors limit events controlled by dephosphorylation, including, but not limited to, transcription factor activation, movement of proteins in or out of the nucleus, proteolysis, and new protein expression. The pelleted cells are then resuspended in ice-cold Hypotonic Buffer causing the cells to swell and increase membrane fragility. Addition of detergent (10% Nonidet P-40) breaks the cell membranes allowing access to the cytoplasmic fraction while maintaining the integrity of the nuclear membrane. After separation of the cytoplasmic fraction from the nuclei by brief centrifugation, the pelleted nuclei are lysed in ice-cold extraction buffer containing a mixture of protease and phosphatase inhibitors. The protein concentration in nuclear and cytoplasmic fractions can then be quantitated using Cayman's Protein Determination Kit (Item No. 704002) or other protein quantification assay. The nuclear and cytoplasmic fractions can be used directly in Cayman's Transcription Factor Assay Kits. To view a schematic of Cayman's Transcription Factor Assays see Figure 1, on page 7.

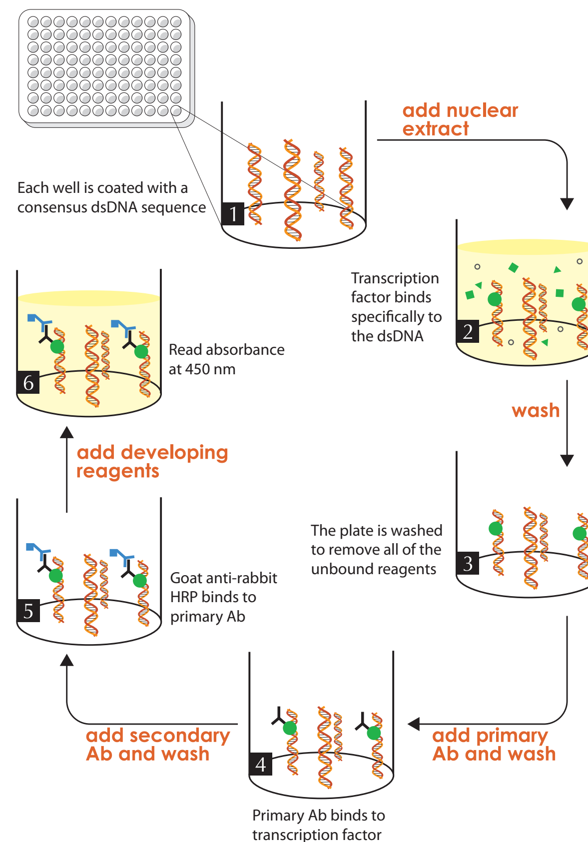

Figure 1. Schematic of the Transcription Factor Binding Assay

Buffer Preparation

1. Nuclear Extraction Protease Inhibitor Cocktail (100X)

Aliquot the Nuclear Extraction Protease Inhibitor Cocktail (100X) (Item No. 10009303) at 50 µl/vial and store at -20°C. This 100X Protease Inhibitor Solution is stable for one year when aliquoted and stored at -20°C.

2. Nuclear Extraction Phosphatase Inhibitors (50X)

This 50X Nuclear Extraction Phosphatase Inhibitor (Item No. 10009305) solution is stable for six months when stored at -80°C.

3. Nuclear Extraction 10 mM Dithiothreitol

One vial (Item No. 10009302) contains 60 µl of 1 M Dithiothreitol (DTT) to be used for preparing the 1X Extraction Buffer. To prepare 10 mM DTT, dilute the 1 M stock solution 1:100 in distilled water. Determine the amount of 10 mM DTT required for your nuclear extractions by using the table, on page 11, for making 1X Extraction Buffer. Adjust the volume according to the total number of plates being analyzed. The 1 M and 10 mM DTT solutions are stable for six months when stored at -20°C.

4. PBS/Phosphatase Inhibitor Solution (1X)

The PBS/Phosphatase Inhibitor Solution is to be used for washing the cell pellet after collection by centrifugation. Determine the amount of PBS/Phosphatase Inhibitor Solution required for your nuclear extractions by using the table below. Adjust the volume according to the total number of plates being analyzed. The phosphatase inhibitors lose activity 24 hours after dilution, therefore any unused PBS/Phosphatase Inhibitor Solution (1X) should be discarded.

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)	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)

5. Hypotonic Buffer (1X)

Determine the amount of Hypotonic Buffer (1X) that will be required for your nuclear extractions by using the table below. Adjust the total volume according to the total number of plates being analyzed. The phosphatase and protease inhibitors lose activity shortly after dilution, therefore any unused 1X Hypotonic Buffer should be discarded.

Reagent	60 mm plate ~3.5×10^6 cells	100 mm plate ~7×10^6 cells	150 mm plate ~1.5×10^7 cells
Nuclear Extraction Hypotonic Buffer (10X) (Item No. 10009301)	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

Table 2. Preparation of Hypotonic Buffer (1X)

6. Extraction Buffer (1X)

Determine the amount of 1X Extraction Buffer that will be required for your nuclear extractions by using the table below. Adjust the volume according to the total number of plates being analyzed. Some of the phosphatase and protease inhibitors lose activity shortly after dilution, therefore any remaining 1X Extraction Buffer should be discarded.

Reagent	60 mm plate ~3.5×10^6 cells	100 mm plate ~7×10^6 cells	150 mm plate ~1.5×10^7 cells
Nuclear Extraction Buffer (2X) (Item No. 10009306)	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

Table 3. Preparation of Extraction Buffer (1X)

ASSAY 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. Prepare 1X PBS/Phosphatase Inhibitor, 1X Hypotonic Buffer, and 1X Extraction Buffer as outlined above in the **Buffer Preparation** section. Adjust the volumes as needed according to the number of cells/plate per prep. A hemocytometer can be used to determine an accurate cell count. Keep all solutions and extracted cytoplasmic and nuclear extracts on ice during the entire protocol.

1. Collect ~10^7 cells in pre-chilled 15 ml tubes. For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Centrifuge suspended cells at 300 x g for five minutes at 4°C.
3. Discard the supernatant. Resuspend 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 one time.
4. Discard the supernatant. Add 500 µl ice-cold 1X Hypotonic Buffer. Mix gently by pipetting and transfer resuspended pellet to a pre-chilled 1.5 ml microcentrifuge tube.
5. Incubate cells on ice for 15 minutes allowing cells to swell.
6. Add 100 µl of 10% Nonidet P-40 Assay Reagent (Item No. 600009 or suitable substitute). Mix gently by pipetting.
7. Centrifuge for 30 seconds (pulse spin) at 4°C in a microcentrifuge. Transfer the supernatant which contains the cytosolic fraction to a new tube and store at -80°C.
8. Resuspend the pellet in 100 µl ice-cold Complete Nuclear Extraction Buffer (1X) (with protease and phosphatase inhibitors). Vortex 15 seconds at highest setting then gently rock the tube on ice for 15 minutes using a shaking platform. Vortex sample for 30 seconds at highest setting and gently rock for an additional 15 minutes.
9. Centrifuge at 14,000 x g for 10 minutes at 4°C. The supernatant contains the nuclear fraction. Aliquot to clean chilled tubes, flash freeze and store at -80°C. Avoid freeze/thaw cycles. The extracts are ready to use in the assay.
10. Keep a small aliquot of the nuclear extract (and cytoplasmic extract) to quantitate 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. Cytoplasmic extracts contain a small amount of detergent which, depending upon the dilution factor, may or may not interfere with the protein quantification assay. It is beneficial to use the 1X Extraction Buffer as the blank and for diluting samples 1:10 and 1:50 prior to quantifying nuclear protein concentrations, use the 1X Hypotonic Buffer without Nonidet P-40 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.

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.
2. While keeping the sample on ice, add 3 ml of ice-cold 1X Hypotonic Buffer supplemented with DTT and Nonidet P-40 (3 µl of 1M DTT and 3 µl of 10% Nonidet P-40) per gram of tissue.
3. Homogenize the sample with a Dounce homogenizer or a polytron device and incubate on ice for 15 minutes.
4. Transfer to prechilled microcentrifuge tubes and centrifuge at 300 x g for 10 minutes at 4ºC and transfer the supernatant into a pre-chilled microcentrifuge tube labeled Tube 1.
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 Hypotonic Buffer by pipetting up and down several times. Transfer to a pre-chilled microcentrifuge tube.
6. Incubate cells on ice for an additional 15 minutes.
7. Add 50 µl of 10% Nonidet P-40. Mix by gently pipetting up and down.
8. Centrifuge at 14,000 x g for 30 seconds (pulse spin) at 4°C in a microcentrifuge. Transfer the supernatant which contains the cytosolic fraction to a new tube and combine with Tube 1 (step #4 above). The supernatant is the cytoplasmic fraction and can be aliquoted and stored at -80°C.

9. Resuspend the pellet in 50 µl ice-cold 1X Extraction Buffer (with protease and phosphatase inhibitors). Vortex 15 seconds at the highest setting and then gently rock the tube on ice for 15 minutes using a shaking platform. Vortex the sample for 30 seconds at the highest setting and gently rock for an additional 15 minutes. Alternatively if a shaker platform is not available, vortex the sample for 30 seconds at the highest setting, place on ice for 10 minutes, repeat the vortex on the highest setting for 30 seconds, place on ice for 10 minutes; repeat four more times for a total of six cycles.
10. Centrifuge at 14,000 x g for 10 minutes at 4°C. The supernatant contains the nuclear fraction. Aliquot to clean chilled tubes, flash freeze and store at -80°C. Avoid freeze/thaw cycles. The extracts are ready to use in the transcription factor assay, or other application.
11. Keep a small aliquot of the nuclear extract (and cytoplasmic extract) to quantitate 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. Cytoplasmic extracts contain a small amount of detergent which, depending upon the dilution factor, may or may not interfere with the protein quantification assay. It is beneficial to use the 1X Extraction Buffer as the blank and for diluting samples 1:10 and 1:50 prior to quantifying nuclear protein concentrations, use the 1X Hypotonic Buffer without Nonidet P-40 as the blank and for diluting cytoplasmic samples.

Steps	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
1. Resuspend and wash cells	PBS/Phosphatase Inhibitors (1X)	3 ml	5 ml	7.5 ml
2. Centrifuge	Centrifuge suspended cells at 300 x g for 5 minutes at 4°C and discard supernatant			
3. Resuspend and wash cells	Repeat steps 1 and 2			
4. Isolation of Cytoplasmic fraction	Hypotonic Buffer (1X)	250 µl	500 µl	1,000 µl
5. Incubate	Incubate cells on ice for 15 minutes allowing the cells to swell			
6. Add Detergent	10% Nonidet P-40	50 µl	100 µl	150 µl
7. Mix	Mix solution gently by pipetting up and down several times			
8. Centrifuge	Centrifuge for 30 seconds (pulse spin) at 4°C in microcentrifuge			
9. Collection of Cytoplasmic fraction	Transfer supernatant (cytoplasmic fraction) to pre-chilled microcentrifuge tube. Store at -80°C. Save small aliquot for protein quantification.			
10. Extraction and solubilization of Nuclear fractions	Extraction Buffer (1X)	50 µl	100 µl	150 µl
11. Resuspend nucleii	Resuspend the pellet in Extraction Buffer.			
12. Nuclear Extraction	Vortex microcentrifuge tube for 15 seconds on highest setting then gently rock on ice for 15 minutes using a platform shaker.			
13.	Repeat step 12			
14. Collection of Nuclear fraction	Centrifuge at 14,000 x g for 10 minutes at 4°C. Transfer supernatant (nuclear fraction) to a pre-chilled microcentrifuge tube. Store at -80°C. Save small aliquot for protein quantification.			

Table 4. Quick protocol (cell extracts)

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. Check that all reagents have been added in the correct volume and in the correct order based upon cell number B. Gently pipette after adding Hypotonic Buffer so that the cell pellet is disrupted C. After adding 10% Nonidet P-40 check lysis using a microscope D. 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. Check that all reagents have been added in the correct volume and in the correct order based upon cell number B. Gently pipette after adding Hypotonic Buffer so that the cell pellet is disrupted C. After adding 10% Nonidet P-40 check lysis using a microscope D. Reduce the number of times the sample is vortexed during lysis E. Reduce the centrifuge time after adding 10% Nonidet P-40 (keep to under 30 seconds) F. Check expiration date on reagents
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

Related Products

ATF2 (Phospho-Thr^{69,71}) Transcription Factor Assay Kit - Item No. 600130
ChREBP Transcription Factor Assay Kit - Item No. 10006909
CREB (Phospho-Ser^{133}) Transcription Factor Assay Kit - Item No. 10009846
HIF-1α Transcription Factor Assay Kit - Item No. 10006910
Liver X Receptor β Transcription Factor Assay Kit - Item No. 10011119
NF-κB (human p50) Transcription Factor Assay Kit - Item No. 10006912
NF-κB (human p50/p65) Combo Transcription Factor Assay Kit - Item No. 10011223
NF-κB (p65) Transcription Factor Assay Kit - Item No. 10007889
Nonidet P-40 Assay Reagent (10%) - Item No. 600009
Nrf2 Transcription Factor Assay Kit - Item No. 600590
Nuclear Extraction Buffer (2X) - Item No. 10009306
Nuclear Extraction Hypotonic Buffer (10X) - Item No. 10009301
Nuclear Extraction PBS (10X) - Item No. 10009304
Nuclear Extraction Phosphatase Inhibitors (50X) - Item No. 10009305
Nuclear Extraction Protease Inhibitor Cocktail (100X) - Item No. 10009303
p53 Transcription Factor Assay Kit - Item No. 600020
p53 Designer Transcription Factor Assay Kit - Item No. 600030
PPARα, δ, γ Complete Transcription Factor Assay Kit - Item No. 10008878
PPARα Transcription Factor Assay Kit - Item No. 10006915
PPARδ Transcription Factor Assay Kit - Item No. 10006914
PPARγ Transcription Factor Assay Kit - Item No. 10006855
Protein Determination Kit - Item No. 704002
SREBP-1 Transcription Factor Assay Kit - Item No. 10010854
SREBP-2 Transcription Factor Assay Kit - Item No. 10007819

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

NOTES

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