MARLIGEN BIOSCIENCES
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Nuclear Extraction Kit

Store at 4°C

Catalog No. 11906-100 100 Reactions

Overview and Intended Use

The Marligen Nuclear Extraction Kit provides material for preparing nuclear extracts from 100 samples having a cell pellet volume of 100ul. The protocol can be scaled to accommodate samples comprising larger or smaller pellet volumes and the number of reactions will vary accordingly.

Principle of Method

Cultured cells are collected by centrifugation and washed in phosphate buffered saline to remove cell culture medium. Cells are then allowed to swell in Complete Hypotonic Cell Lysis Buffer and lysis is facilitated by the addition of Detergent Solution. The cell nuclei are collected by gentle centrifugation, and the cytoplasm is removed and can be stored at -80°C for future analysis. The nuclear pellet is washed twice in Complete Nuclear Wash Buffer and extracted by addition of Complete Extraction Buffer 1 and Complete Extraction Buffer 2, and incubated on ice for 30 minutes. The nuclear extract is clarified by centrifugation, and stored at -80°C until use.

Important Information

READ ENTIRE PROTOCOL BEFORE USE

To avoid introducing additional variables that could affect experimental results, the reagents supplied in this kit are prepared without preservatives. Therefore, we recommend the use of sterile technique and pipettes with these reagents to minimize the potential for microbial contamination. Maintain reagents on ice during the procedure, and promptly return unused reagents to 4°C storage conditions upon completion of the work.

Safety and Use Statement

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

Components included with this kit:

Hypotonic Lysis Buffer	50 ml
Wash Solution	100 ml
Extraction Buffer 1	5 ml
Extraction Buffer 2	5 ml
Detergent Solution	5 ml

Storage Conditions

Store all reagents at 4°C when not in use and keep on ice during preparation of extracts.

Materials and equipment required but not supplied:

Phosphatase Inhibitor cocktail (Sigma Catalogue no. P5726)
Protease Inhibitor cocktail (Sigma Catalogue no. P8340)
0.1 M DTT (dithiothreitol, Sigma Catalogue no. D9779) in deionized water (store @ -20ºC)
100 mM PMSF (Sigma P7626) in 100% ethanol (store @ -20ºC)
Cold Phosphate Buffered Saline without Calcium or Magnesium, pH 7.4
Cold Phosphate Buffered Saline with 5mM Sodium EDTA, pH 7.4 (without Calcium or Magnesium)
Cold 5ml, 15ml, or 50ml conical tubes
Cold Microcentrifuge tubes
Refrigerated microcentrifuge

Set Up

Calculate the number of tubes required (two per sample) and chill by placing on ice for at least 15 minutes.
Remove reagents from refrigeration and place on ice. Keep all kit reagents and prepared reagents on ice throughout this procedure.
Calculate the approximate quantity required for Hypotonic Lysis Buffer, Extraction Buffer 1, Extraction Buffer 2, and Wash Solution (see steps 7, 11, and 14).
Prechill an appropriate container for each reagent by placing on ice. Thaw protease inhibitors, DTT, and PMSF on benchtop.
NOTE: DO NOT DILUTE THE SOLUTIONS AT THIS TIME. DILUTE IMMEDIATELY PRIOR TO USE.

Protocol For Suspension Cells

NOTE: All steps should be performed on ice or in a refrigerated microcentrifuge.

1. Determine cell density in liquid media.
2. Aliquot up to 2×10^7 cells per tube into 15 ml conical tubes.
3. Collect the cells by centrifugation at 800 x g for 5 minutes at 4°C. Remove supernatant.
4. Add 10ml cold PBS, resuspend cells and pellet by centrifugation. Remove supernatant. Repeat for a total of 2 PBS washes.
5. Pellet cells by centrifugation 800 X g for 5 minutes in a refrigerated centrifuge.
6. Remove the supernatant slowly. Do not disturb the pellet. Discard the supernatant. Place the tube containing the pellet on ice. Estimate the volume of each pellet.
7. Immediately prior to use, prepare Complete Hypotonic Lysis Buffer in the prechilled container according to the chart below. Five (5) pellet volumes of Complete Hypotonic Lysis Buffer will be used for each sample. A minimum of 500 µl will be used for cell pellets of less than 100 µl. Volumes can be directly scaled to accommodate larger pellet volumes based on the minimum requirement of 500µl Hypotonic Lysis Buffer for 100µl of cell pellet.

Reagent	Quantity needed for 500 µl
Hypotonic Lysis Buffer	500 µl
Protease Inhibitor	5 µl
Phosphatase Inhibitor	5 µl
0.1 M DTT	5 µl
0.1 M PMSF	0.5 µl

Place Complete Hypotonic Lysis Buffer on ice.

NOTE: The Complete Hypotonic Lysis Buffer should be used within 10 minutes of preparation.

8. Add 500 µl or 5 pellet volumes (whichever is greater) of Complete Hypotonic Lysis Buffer to each cell pellet. Resuspend each pellet by pipeting up and down and transfer to clean prechilled 1.5 ml microcentrifuge tubes. Incubate on ice for 10 minutes.
9. Add 5 µl of Detergent Solution for each 100 µl of Complete Hypotonic Lysis Buffer used in step 8. Vortex for 5 seconds and centrifuge for 5 minutes at 800 x g (~3000 rpm) in a refrigerated microcentrifuge. During this centrifugation step prepare Complete Wash Solution as outlined in Step 11.

10. Remove supernatant from the nuclear Pellet. This supernatant contains **the cytoplasmic extracts** that can be stored at –80ºC, if desired.
11. Immediately prior to use, prepare Complete Wash Solution in the prechilled container. Each sample will require 1ml of Complete Wash Solution.

Reagent	Quantity needed for each sample
Wash Solution	1000 µl
Protease Inhibitor	10 µl
Phosphatase Inhibitor	10 µl
0.1 M DTT	10 µl
0.1 M PMSF	1 µl

12. Add 500ul of cold Complete Wash Solution to each nuclear pellet and mix by gently pipetting up and down. Centrifuge for 5 minutes at 800 x g (~3000 rpm) in a refrigerated microcentrifuge and discard supernatant. Estimate and record pellet volume. **IMPORTANT: This estimated pellet volume will be used to calculate the amount of complete Extraction Buffer 1 and Extraction Buffer 2 required for Step 13 below.**
13. Repeat the Wash step as described in step 12 until the supernatant is clear. During the centrifugation step, Complete Extraction Buffers 1 and 2 should be prepared. Use the pellet volumes estimated in step 9 to prepare Complete Extraction Buffers 1 and 2 as described in the table. Complete Extraction Buffers 1 and 2 should be prepared in **prechilled** containers immediately prior to use.

Complete Extraction Buffer 1		Complete Extraction Buffer 2	
Reagent	Quantity for 200 µl nuclear pellet	Reagent	Quantity for 200 µl nuclear pellet
Extraction Buffer 1	100 µl	Extraction Buffer 2	100 µl
Protease Inhibitor	1µl	Protease Inhibitor	1µl
Phosphatase Inhibitor	1µl	Phosphatase Inhibitor	1µl
0.1 M DTT	1µl	0.1 M DTT	NONE
0.1 M PMSF	0.1µl	0.1 M PMSF	0.1µl

14. Add one-half (½) pellet volume (~10 µl for 5 x 10^6 cells) of **Complete Extraction Buffer 1** followed by ½ pellet volume (~ 10 µl for 5 x 10^6 cells) of **Complete Extraction Buffer 2** to each cell pellet. Vortex immediately for 5 seconds.
15. Incubate on ice for 30 minutes. Vortex momentarily at high speed every 10 minutes.
16. Centrifuge at high speed for 30 minutes in a refrigerated microcentrifuge.
17. Remove and save supernatant in a chilled tube. **This is the nuclear extract**.
18. Quantitate extracts prior to freezing. (The Bradford assay is the recommended method).
19. Dilute the extracts to the desired concentration (5- 10 µg/µl is recommended) in Extract Dilution Buffer prepared according to the table below.

Buffer	To Prepare 100 µl	To Prepare 200 µl	To Prepare 500 µl
Wash Solution	50 µl	100 µl	250 µl
Extraction Buffer 1	25 µl	50 µl	125 µl
Extraction Buffer 2	25 µl	50 µl	125 µl

20. Store extracts at –70ºC to –80ºC

Protocol for Adherent Cells

<u>Note: Keep cells on ice or refrigerated for this entire procedure.</u>

1. Prepare Complete Hypotonic Lysis Buffer in a pre-chilled container (see table supplied in step 7 of the protocol for Suspension Cells). The total volume of Hypotonic Lysis Buffer required for each sample can be calculated according to the following table:

Culture Vessel	Volume of Complete Hypotonic Lysis Buffer
6-well plate	500 µl/well
100mm X 20 mm Dish	2 ml
T-25 Flask	500 µl
T-75 Flask	2 ml
T-175 Flask	4 ml

2. Remove cell culture media by aspiration and wash cells twice with 2-10ml of ice-cold PBS (not containing calcium or magnesium). It is important to remove all PBS from the cultured cells before proceeding, but do not let the cells dry.
3. Add Complete Hypotonic Lysis Buffer making sure that it is distributed across the entire surface of the culture well or flask.
4. Collect cells with a cell scraper or rubber policeman and transfer to an appropriately sized centrifuge tube (microcentrifuge tube or 15ml conical tube).
5. Incubate on ice for 10 minutes. Continue with Step 9 for the Protocol for Suspension Cells.