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A Geno Technology, Inc. (USA) brand name

Nuclear & Cytoplasmic Extraction

(Cat. # 786-182)

INTRODUCTION

The Nuclear & Cytoplasmic Extraction Kit is useful for the enrichment of cytoplasmic and nuclei fractions from cultured cells and tissues for expression of proteins and transport studies as well as proteomic analysis. This kit is based on organic buffers and contains a proprietary combination of various salts and agents. Depending on application, additional agents such as reducing agents and protease inhibitors may be added into the buffer. The kit is provided with reagents for solubilization of nuclear fraction.

The kit is supplied with enough reagents to purify 300 preps, where a prep is a 20µl wet cell pellet of 2 x 10^6 cells or 200 preps of 20mg tissue.

ITEM(S) SUPPLIED (Cat. # 786-182)

Description	Size
SubCell Buffer-I	60ml
SubCell Lysis Reagent	3ml
Nuclear Extraction Buffer	12ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store the kit components at 4°C upon arrival. The kit is stable for one year when stored unopened. Use aseptic techniques when handling the reagent solutions.

ADDITIONAL ITEMS REQUIRED

- Centrifuge and centrifuge tubes,
- PBS
- Protease Inhibitor Cocktail, we recommend ProteaseArrest™ Protease Inhibitor Cocktail (Cat. # 786-711)

PREPARATION BEFORE USE

1. All buffers should be kept ice cold.

2. All centrifugation steps should be performed at 4°C.

3. Add appropriate protease inhibitor cocktail (e.g. G-Biosciences' ProteaseArrest, Cat. # 786-108) to SubCell Buffer-I just before use.

PROTOCOLS

A. Cultured Cells Preparation

This protocol is for processing 1-10 x 10^6 cells. It can be scaled up and down accordingly.

1. ***Adherent Cells:*** Harvest adherent cells with trypsin-EDTA and centrifuge at 500 x *g* for 5 minutes.
   Suspension Cells: Harvest cells by centrifuging at 500 x *g* for 5 minutes.
2. Carefully remove and discard the supernatant. Wash the cell pellet with 1ml ice cold PBS.
3. Transfer 1-10 x 10^6 cells to a 1.5ml centrifuge tube and pellet cells at 500 x *g* for 5 minutes. Carefully remove all the supernatant with a pipette leaving cell pellet as dry as possible.
4. Add an appropriate volume of ice cold SubCell Buffer-I as indicated in Table 1.

Table 1: Volume of reagents for wet cell pellet volumes.

Wet Cell Pellet Volume (µL)	SubCell Buffer-I (µl)	SubCell Lysis Reagent (µl)	Nuclear Extraction Buffer (µl)
10	100	5	20
20	200	10	40
50	500	25	100
100	1000	50	200

5. Proceed to Nuclear & Cytoplasmic Extraction section.

B. Tissue Preparation

OPTIONAL: *Delipidated BSA can be added to 1X SubCell Buffer-II to the concentration of 2mg/ml for removing free fatty acids prior processing. An appropriate amount protease inhibitor cocktail also can be added to the 1X SubCell Buffer-II just before use.*

1. Use a fresh tissue sample (obtained within one hour of sacrifice) kept on ice. Do not freeze.
2. Weigh approximately 20-100mg tissue. On a cooled glass plate, with the aid of a scalpel, mince the tissue into very small pieces.
3. Wash the tissue with 1ml ice cold PBS and centrifuge at 500 x *g* for 5 minutes. Carefully remove all the supernatant with a pipette leaving tissue pellet as dry as possible.
4. Homogenize the tissue in a Dounce homogenizer or tissue grinder with an appropriate volume of SubCell Buffer-I (Table 2).

Table 2: Volume of reagents for tissue weight.

Tissue Weight (mg)	SubCell Buffer-I (µl)	SubCell Lysis Reagent (µl)	Nuclear Extraction Buffer (µl)
25	250	12.5	50
50	500	25	100
75	750	37.5	150
100	1000	50	200

6. Proceed to Nuclear & Cytoplasmic Extraction section.

C. Nuclear & Cytoplasmic Extraction

NOTE: Scale the volumes used according to the table 1 and 2. Ensure the ratios of SubCell Buffer-I : SubCell Lysis reagent : Nuclear Extraction Buffer remain as 100:5:20 respectively.

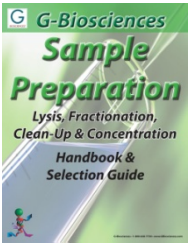
1. Vigorously vortex the tube for 15 seconds to completely resuspend and disperse the cell pellet. Incubate on ice for 10 minutes.
2. Add the appropriate volume of SubCell Lysis Reagent to the tube.
3. Immediately vortex the tube for 5 seconds at maximum setting.
4. Incubate on ice for 1 minute and vortex as in step 3.
5. Centrifuge the tube for 5 minutes at maximum speed (~16,000 x *g*).
6. Immediately, transfer the supernatant, the cytosol, to a clean chilled tube and incubate on ice. Store at -80°C for long term storage.
7. Resuspend the nuclear pellet from step 6 in ice cold Nuclear Extraction Buffer.
8. Vigorously vortex for 15 seconds. Incubate on ice for 30 minutes, repeating the 15 second vortex every 10 minutes.
9. Centrifuge the tube for 10 minutes at maximum speed (~16,000 x *g*). Immediately, transfer the supernatant, the nuclear protein fraction, to a clean chilled tube and incubate on ice. Store at -80°C for long term storage.

TROUBLESHOOTING

Issue	Possible Reason	Suggested Resolution
Poor separation of nuclear and cytosolic proteins	Poor cell lysis	Ensure all residual PBS is removed prior to addition of SubCell Buffer-I
		Extend vortex time and use maximum vortex speed to ensure cells fully dispersed
		Increase incubation times
	Cytoplasmic fraction not completely removed	Ensure all supernatant is removed from nuclear pellet prior to its lysis
		Briefly centrifuge nuclear pellet after supernatant is removed to collect any excess supernatant
		Wash nuclear pellet with SubCell Buffer-I or PBS
	Poor homogenization of tissue	Optimize tissue homogenization
Low yield of nuclear protein	Cell pellet was incompletely dispersed	Extend vortex time and use maximum vortex speed
	Incomplete isolation of nuclei	Increase the centrifugation time following addition of SubCell Lysis Reagent
Low yield of cytoplasmic proteins	Cell pellet was incompletely dispersed	Extend vortex time and use maximum vortex speed
	Cells were not completely lyzed	Increase volume of SubCell Lysis Reagent
Low total protein concentration	Volumes used were inappropriate for wet cell pellet volume or tissue weight	Ensure appropriate volumes are used as indicated in Table 1 and 2
Proteins have low or no activity	Proteins degraded by cellular proteases	Use a Protease Inhibitor Cocktail, we recommend ProteaseArrest™ Protease Inhibitor Cocktail
	Proteins degraded	Ensure buffers are pre-chilled and all incubations and centrifugations are performed at 4°C

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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