YamayBio

Animal Nuclear and Cytoplasmic Extraction Kit (High-Efficiency for WB)

QUICK START GUIDE

For research use only.
Not for use in diagnostic procedures.

Contents and Storage

Animal Nuclear and Cytoplasmic Extraction Kit

BF7476 (High-Efficiency for WB) 50 Samples

Storage: Store at 4 °C for 12 months. The product is shipped with ice packs.

Component	Cat. No.	Volume
NCE Reagent A	BF7401	20 mL
NCE Reagent B	BF7402	0.5 mL
NCE Reagent C	BF7403	5 mL

Introduction

This kit enables rapid and efficient separation of nuclear and cytoplasmic proteins from animal cells or tissues. Through stepwise cell lysis, intact nuclei are first isolated from the cytoplasm, followed by extraction of nuclear proteins. The formulation contains powerful detergents that allow the extraction of nuclear membrane proteins, histones, and soluble transcription factors. Compared with traditional methods requiring 30–40 minutes, this kit completes nuclear protein extraction in approximately 10 minutes while maintaining clear nuclear–cytoplasmic separation. It is particularly suitable for Western blotting applications.

Additional Material Required

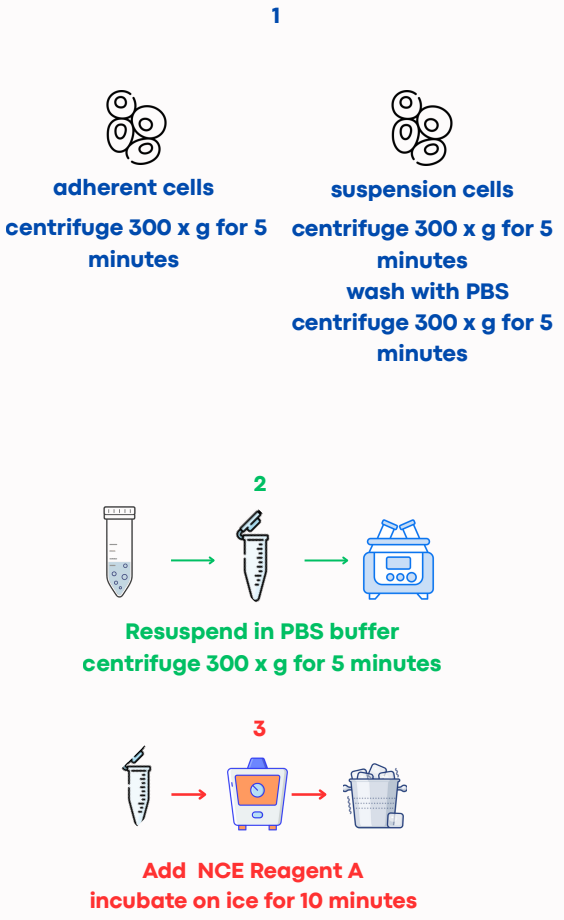
1. Protease inhibitors (e.g., Cat. No. BH5481).
2. Protease and phosphatase inhibitors (e.g., Cat. No. BH5483).
3. For tissue samples, a 2 mL Dounce Tissue Homogenizer is required.

Important Product Information

1. This kit is intended for use with fresh (not frozen) cells or tissue samples. Use protease inhibitors to maintain extract integrity and function. Immediately before use, add protease inhibitors to NCE Reagent A and NCE Reagent C from concentrated stocks (e.g., 100X) to minimize reagent dilution. It is unnecessary to add protease inhibitors to NCE Reagent B.
2. Perform all centrifugation steps at 4°C. Keep cell samples and extracts on ice.

Quick Protocol

Protocol 1: Cell Culture Preparation

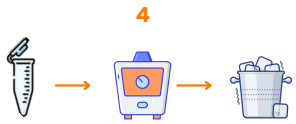


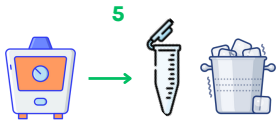
1. For **adherent cells**, wash with PBS buffer, then use a **cell scraper to detach the cells** from the culture dish. Resuspend the cells and transfer them to a centrifuge tube. **Centrifuge at 300 × *g* for 5 min** and discard the supernatant. For **suspension cells**, transfer cells directly to a centrifuge tube and **centrifuge at 300 × *g* for 5 minutes**. Remove the supernatant, resuspend the pellet in PBS, **centrifuge again at 300 × *g* for 5 minutes**, and discard the supernatant.
2. Resuspend the cells in PBS. Transfer 1-10 × 10^6 cells into a 1.5 mL microcentrifuge tube and **centrifuge at 300 × *g* for 5 minutes**. Discard the supernatant, leaving the pellet as dry as possible.
3. Add ice-cold **NCE Reagent A** according to Table 1. Vortex vigorously and **incubate it on ice for 10 minutes**. Proceed with the experiment, following the reagent volumes indicated in Table 1.

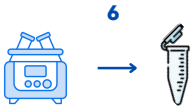
Table 1. Reagent volumes for different packed cell volumes.*

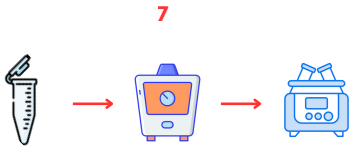
Packed Cell Volume (µL)	NCE Reagent A (µL)	NCE Reagent B (µL)	NCE Reagent C (µL)
10	100	5	50
20	200	10	100
50	500	25	250
100	1000	50	500

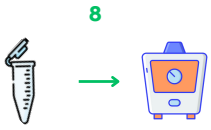
*For HeLa cells, 2 × 10^6 cells is equivalent to 20µL packed cell volume.

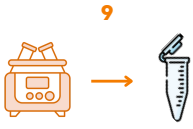












4. Add ice-cold **NCE Reagent B** to the tube. **Vortex at the highest setting for 5 seconds** and **incubate on ice for 1 minute**.

5. **Vortex again for 5 seconds** and **incubate the tube on ice for 2 minutes**.

6. **Centrifuge at 1,600 × *g* for 10 minutes at 4 °C**. Immediately transfer the supernatant (cytoplasmic fraction) to a clean, pre-chilled tube and place on ice or store at –80 °C.

7. Resuspend the pellet in the same volume of **NCE Reagent A**, Vortex immediately for 15 seconds and **centrifuge for 5 minutes at 13,000 × *g* at 4 °C**. Discard the supernatant.

8. Add **NCE Reagent C** to the pellet. **Vortex at maximum speed for 15 seconds**, place on ice, and **vortex again every 10 minutes** for a **total** incubation of **40 minutes**.

9. **Centrifuge at 13,000 × g for 10 minutes at 4 °C**. Transfer the supernatant containing nuclear proteins to a new tube and place on ice.

10. Store extracts at -80°C until use.

Protocol 2: Tissue Preparation

1

20-100mg tissue centrifuge 500 x g for 3 minutes

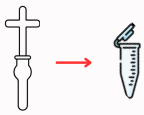
1. Cut 20-100 mg of tissue into small pieces and place in a 2 mL microcentrifuge tube. Wash with PBS and **centrifuge at 500 × *g* for 3 minutes at 4°C**.

2

Discard the supernatant

2. Carefully discard the supernatant, leaving the cell pellet as dry as possible.

3



Add NCE Reagent A incubate on ice for 15 minutes

3. Homogenize tissue using a Dounce homogenizer in the appropriate volume of **NCE Reagent A** (Table 2). Proceed with nuclear and cytoplasmic protein extraction using the reagent volumes indicated.

Table 2. Reagent volumes for different tissue amounts.*

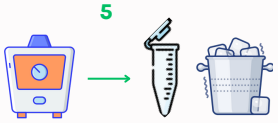
Tissue Weight (mg)	NCE Reagent A (µL)	NCE Reagent B (µL)	NCE Reagent C (µL)
20	200	10	100
40	400	22	200
80	800	40	400
100	1000	50	500

*Different tissue types may require more or less NCE Reagent C per weight to optimally extract cytoplasmic and nuclear proteins.

4

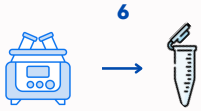
Add NCE Reagent B
incubate on ice for 1 minutes

5



Vortex 5 seconds
incubate on ice for 2 minutes

6

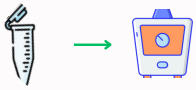


Centrifuge 1,600 x g for 10 minutes at 4 °C
transfer supernatant

7

Add NCE Reagent A
Vortex 15 seconds
Centrifuge 13,000 x g for 5 minutes at 4 °C

8



Add NCE Reagent C vortex 15 seconds
vortex every 10 minutes for 40 minutes

9

Centrifuge 13,000 x g for 10 minutes at 4 °C
transfer supernatant

10

Store at -80°C

4. Add ice-cold **NCE Reagent B** to the tube. **Vortex the tube for 5 seconds** on the highest setting. **Incubate the tube on ice for 1 minute**.

5. **Vortex the tube for 5 seconds** on the highest setting. **Incubate the tube on ice for 2 minutes**.

6. **Centrifuge** the incubated cells **for 10 minutes at 1,600 × *g*, 4 °C**. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled tube. Place this tube on ice until use or store at –80 °C.

7. Resuspend the pellet in the same volume of **NCE Reagent A**, Vortex on the highest setting for 15 seconds, and immediately **centrifuge for 5 minutes at 13,000 × *g*, 4 °C**. Discard the supernatant.

8. Add **NCE Reagent C** to the pellet, and **vortex at maximum speed for 15 seconds**. Place the suspension on ice, and **vortex again every 10 minutes** during a **total** incubation of **40 minutes**.

9. **Centrifuge the suspension for 10 minutes at 13,000 × g, 4 °C**. Transfer the supernatant containing soluble nuclear proteins to a new tube. Place on ice.

10. Store extracts at -80°C until use.