ClearBand Cytoplasmic & Nuclear Protein Fractionation Kit

from Cultured Cells and Tissue Samples

100 extraction from 100 cell pellet fractions having packed cell volumes of 20 µL each or 20 mg tissue each

Cat No: CNF100

Shipping : Ship with blue ice.
Storage : Stable for 1 year at 4°C.

General Information

ClearBand Cytoplasmic & Nuclear Protein Fractionation Kit (from Cultured Cells and Tissue Samples) is formulated for stepwise separation and preparation of cytoplasmic and nuclear extracts from mammalian cultured cells or tissue. The extracted proteins are suitable for SDS-PAGE, Western blot, ELISA, immunoprecipitation, transcription factor- and enzyme activity assays. Extracts obtained with *ClearBand* Cytoplasmic & Nuclear Protein Fractionation Kit generally have less than 10% contamination between nuclear and cytoplasmic fractions, which is sufficient purity for most experiments involving nuclear extracts.

ClearBand Cytoplasmic & Nuclear Protein Fractionation Kit is intended for use with fresh cells or tissue samples.

Important Note: Use protease inhibitors to maintain extract integrity and function. Immediately before use, add protease inhibitors to CER I and NER I from concentrated stocks (e.g., 100X) to minimize reagent dilution. It is unnecessary to add protease inhibitors to CER II and NER II.

Important Note: If large volumes of nuclear extract are required in subsequent applications or if problems occur with downstream assays, dialyze the nuclear extract to remove excess salts before use. The detergent in the reagents is not dialyzable, but it will be primarily in the cytoplasmic fractions.

Important Note: Perform all centrifugation steps at 4°C. Keep cell samples and extracts on ice.

Protocol (For cultured cells)

Each procedure is suitable for extraction from up to $1x10^6$ cells. If extraction of cytoplasmic and nuclear protein from more tissue is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. For adherent cells, harvest with trypsin-EDTA and then centrifuge at 800 rpm for 5 minutes. For suspension cells, harvest by centrifuging at 800 rpm for 5 minutes. Prepare approximately $1x10^6$ cells per extract.

2. Wash cells gently with 1x PBS buffer (not provided). Collect the cells by centrifugation using a microcentrifuge at 800 rpm for 5 minutes.

3. Use a pipette to carefully remove and discard the supernatant, leaving the pellet as dry as possible.

4. Resuspend the pellet in 5 volumes (approximately 100 µL) of ice-cold *ClearBand* CE I Buffer to the cell pellet.

Note: Add 1% protease inhibitors (v/v) *ClearBand* CER I from concentrated stocks (e.g., 100X) prior to use.

5. Vortex the tube vigorously on the highest setting for 15 seconds to obtain a homogeneous cell suspension. Incubate on ice for 10 minutes.

6. Vortex the tube vigorously on the highest setting for 5 seconds. Centrifuge the tube at 12000 rpm for 5 minutes.

7. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled microcentrifuge tube. Place this tube on ice until use or store aliquots at -80°C for future use.

For further information;
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8. Add ice-cold 100 µL *ClearBand* CE II Buffer to the insoluble debris containing nuclei produced in step 6.

9. Centrifuge the tube at 5000 rpms for 10 minutes and discard the supernatant.

10. Repeat steps 8 and 9 twice. Remove the supernatant leaving the cell pellet as dry as possible.

11. Add ice-cold 50 µL *ClearBand* NE I Buffer to the insoluble pellet containing nuclei.

Note: Add 1% protease inhibitors (v/v) *ClearBand* NER I from concentrated stocks (e.g., 100X) prior to use.

12. Add 20 µl of *ClearBand* NE II Buffer. Place the tube in a homogenizer with a stainless-steel bead (5 mm in diameter) for 5 minutes.

13. Vortex on the highest setting for 5 seconds to obtain a homogeneous suspension (If not complete, prolong the vortex time). Place the homogeneous suspension on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 30 minutes.

14. Centrifuge the tube at 12000 rpms for 10 minutes.

15. Immediately transfer the supernatant containing nuclear proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.

Protocol (For tissue samples)

Each procedure is suitable for extraction from up to 20 mg fresh tissue samples. If extraction of cytoplasmic and nuclear protein from more tissue is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. Cut 20 mg of tissue into small pieces and place in a microcentrifuge tube.

2. Wash tissue pieces gently with 1x PBS buffer (not provided). Collect the tissue material by centrifugation using a microcentrifuge at 800 rpm for 5 minutes.

3. Use a pipette to carefully remove and discard the supernatant, leaving the tissue pellet as dry as possible.

4. Homogenize tissue using a Dounce homogenizer or a tissue grinder in 5 volumes (approximately 100 µL) of ice-cold *ClearBand* CE I Buffer.

Note: Add 1% protease inhibitors (v/v) *ClearBand* CER I from concentrated stocks (e.g., 100X) prior to use.

5. Vortex the tube vigorously on the highest setting for 15 seconds to obtain a homogeneous suspension. Incubate on ice for 10 minutes.

6. Vortex the tube vigorously on the highest setting for 5 seconds. Centrifuge the tube at 12000 rpm for 5 minutes.

7. Immediately transfer the supernatant containing cytoplasmic proteins to a clean pre-chilled microcentrifuge tube. Place this tube on ice until use or store aliquots at -80°C for future use.

8. Add ice-cold 100 µL *ClearBand* CE II Buffer to the insoluble debris containing nuclei produced in step 6.

9. Centrifuge the tube at 5000 rpms for 10 minutes and discard the supernatant.

10. Repeat steps 8 and 9 twice. Remove the supernatant leaving the pellet as dry as possible.

11. Add ice-cold 50 µL *ClearBand* NE I Buffer to the insoluble pellet containing nuclei.

Note: Add 1% protease inhibitors (v/v) *ClearBand* NER I from concentrated stocks (e.g., 100X) prior to use.

12. Add 20 µl of *ClearBand* NE II Buffer. Place the tube in a homogenizer with a stainless-steel bead (5 mm in diameter) for 5 minutes.

13. Vortex on the highest setting for 5 seconds to obtain a homogeneous suspension (If not complete, prolong the vortex time). Place the homogeneous suspension on ice and continue vortexing for 15 seconds every 10 minutes, for a total of 30 minutes.

14. Centrifuge the tube at 12000 rpms for 10 minutes.

15. Immediately transfer the supernatant containing nuclear proteins to a clean pre-chilled tube. Place this tube on ice until use or store aliquots at -80°C for future use.

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