# Nuclear and Cytoplasmic Protein Extraction Kit

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Nuclear and Cytoplasmic Protein Extraction Kit is a simple and convenient method for extracting and separating nuclear and cytoplasmic proteins in 90 minutes. It is designed to quickly prepare highly enriched fractions of cytoplasmic and nuclear proteins from eukaryotic samples, such as cultured cells and fresh tissues. Extracted proteins can be directly used for other applications, such as Western Blot, EMSA, and measurement of enzyme activity. One kit (50 tests) can be used for up to 50 samples (60 mg /sample, or 2 ×$10^6$ HeLa cells).

### Kit Components:

- Cytoplasmic Protein Extraction Reagent A (CER A): 10 ml
- Cytoplasmic Protein Extraction Reagent B (CER B): 1 ml
- Nuclear Protein Extraction Reagent (NER): 3 ml

**Tested Applications:** WB

**Storage:** Store all reagents at -20 °C for up to one year.

**Note:** THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.




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**Directions for use:** Before carrying out the procedure, thaw all reagents, then place on ice. Gently shake each reagent bottle to ensure thorough mixing. Prepare a working CER A solution by taking an appropriate volume of CER A and add PMSF to a final concentration of 1 mM. Prepare a working NER solution by taking an appropriate volume of NER and add PMSF to a final concentration of 1 mM. The working solutions should be prepared 2-3 minutes before the carrying out the protocol below.

**Cultured Cell Samples**

1. For **adherent** cells: wash with PBS and scrape to collect the cells; or treat cells with EDTA and collect cells with a pipette. Centrifuge for several minutes. Discard the supernatant and collect the cell pellets for future use. Do not digest with pancreatin to avoid protein degredation.
For **suspension** cells: wash with PBS, then centrifuge for several minutes. Discard the supernatant and collect the cell pellets for future use.
2. Add 200 µl of working CER A solution (containing PMSF) per 20 µl of cell pellets (approx 2 × $10^6$ cells, or 40 mg or 40 mg).
3. Vortex at maximum speed for 5 seconds to suspend the cell pellets. The vortex time can be increased to ensure complete suspension.
4. Leave on ice for 10-15 minutes.
5. Add 10 µl of CER B, vortex at maximum speed for 5 seconds, then leave on ice for 1 minute.
6. Vortex at maximum speed for 5 seconds, then centrifuge at 12,000-16,000 × g at 4 °C for 5 minutes.
7. Immediately pipette the supernatant (containing cytoplasmic proteins) into a cold tube. The precipitate should not be disturbed. Assay cytoplasmic proteins immediately or store at -70 °C for future analysis.
8. For the remaining precipitate, discard the supernatant to avoid contamination with cytoplasmic proteins. Add 50 µl of working NER solution (containing PMSF) to the precipitate.
9. Vortex at maximum speed for 15-30 seconds to suspend the cell pellets, then leave on ice for 1-2 minutes. Repeat this for 30 minutes.
10. Centrifuge at 12,000-16,000 × g at 4 °C for 5 minutes.
11. Immediately pipette the supernatant (containing nuclear proteins) into a cold tube. The precipitate should not be disturbed. Assay cytoplasmic proteins immediately or store at -80 °C for future analysis.

**Fresh Tissue Samples**

1. In a separate tube, add CER A and CER B at a ratio of 20:1 (e.g. add 200 µl of CER A and 10 µl of CER B). Mix thoroughly. Add PMSF to a final concentration of 1 mM to prepare a working tissue homogenate solution.
2. Cut the tissue into small slices. Add 200 µl of working tissue homogenate solution for each 60 mg of tissue. Homogenise completely on ice or at 4 °C with a glass homogeniser.
3. Transfer the homogenate to a new tube and leave on ice for 15 minutes.
4. Centrifuge at 1500 × g at 4 °C for 5 minutes.
5. Immediately pipette the supernatant (containing cytoplasmic proteins) into a cold tube. The precipitate should not be disturbed. Assay cytoplasmic proteins immediately or store at -70 °C for future analysis.
6. Add 200 µl of working CER A solution (containing PMSF) per 20 µl of precipitate.
7. Vortex at maximum speed for 5 seconds to suspend the cell pellets. The vortex time can be increased to ensure complete suspension.
8. Leave on ice for 10-15 minutes.
9. Vortex at maximum speed for 5 seconds, then centrifuge at 12,000-16,000 × g at 4 °C for 5 minutes.
10. Immediately pipette the supernatant (containing cytoplasmic proteins) into a cold tube. The precipitate should not be disturbed. The cytoplasmic proteins obtained in this step can be combined with those obtained in step 5. Assay cytoplasmic proteins immediately or store at -70 °C for future analysis.
11. For the remaining precipitate, discard the supernatant to avoid contamination with cytoplasmic proteins. Add 50 µl of working NER solution (containing PMSF) to the precipitate.
12. Vortex at maximum speed for 15-30 seconds to suspend the cell pellets, then leave on ice for 1-2 minutes. Repeat this for 30 minutes.
13. Centrifuge at 12,000-16,000 × g at 4 °C for 5 minutes.
14. Immediately pipette the supernatant (containing nuclear proteins) into a cold tube. The precipitate should not be disturbed. Assay cytoplasmic proteins immediately or store at -80 °C for future analysis.

**Notes**

• Phenylmethylsulfonyl fluoride (PMSF) is not included in this kit. It can be purchased separately (abx090634).
• All protein extraction steps should be carried out on ice or at 4 °C.
• This kit is intended for use with cultured cell and fresh tissue samples. It is not suitable for use with frozen tissue samples.
• Personal Protective Equipment (PPE), such as lab coats, disposable gloves and lab glasses, should be worn when using this kit.




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