BIO BASIC
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Product information

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Membrane, Nuclear & Cytoplasmic Protein Extraction Kit

Catalog #: BSP002
Size: 50prep
Storage: 4°C (Solution A, B, C), -20°C (all other components)

Product Description:

Membrane, Nuclear and Cytoplasmic Protein Extraction Kit is designed for efficient separation of membrane, nuclear and cytoplasmic fractions with little or no crosscontaminations. The extracted membrane, nuclear and cytoplasmic proteins are functional and compatible with many downstream assays such as transcriptional activity, RNA splicing, gel shift assay, reporter assays, immunoprecipitation, enzyme activity assays, Western blotting, ELISA and so on . The kit offers additional protease inhibitor cocktail and phosphatase inhibitor cocktail to maintain protein integrity and high biology activity. The kit is sufficient for 50X10^7 cells or 50x100mg tissue samples.

Components:

Name	Size
Solution A	80ml
Solution B	30ml
Solution C	30ml
DTT Solution	150ul
Protease Inhibitor	150ul
Phosphatase Inhibitor	750ul
PMSF	1500ul

Shipping and Storage:

Kit shipped at ambient temperature. Upon receipt store Solution A, B, C at 4°C. Store other components at -20°C. Keep away from light.

Procedure:

1. Harvest about 1x10^7 cell from cell suspension or Monolayer (Adherent) Cultures. Wash the cells three times with 0.5ml ice cold PBS (not provided in the kit).

Note: For tissue samples, weigh 200mg of tissue. Try to remove fat and nerve tissue as much as possible and then cut tissue into small pieces with a scalpel knife.

2. Before use, add **1ul** Protease inhibitor, **5ul** Phosphatase inhibitor, **1ul** DTT, and **10ul** PMSF into 1ml Solution **A**. Mix well and keep on ice.

Procedure Continued:

3. Add 1ml ice cold Solution **A** to the sample from step **1**. Vortex. Homogenize the sample with glass homogenizer for 30-50 intervals or sonicate the sample for 30 seconds with 1minutes interval. Repeat operation three times. Check efficiency of homogenization and make sure no existing cell clumps.

4. Vortex mixture vigorously for 10 seconds. Incubate at 4°C(on ice) for 20 minutes. Shake occasionally 3-5 times during incubation, then centrifuge at 12000rpm for 10 minutes. Transfer supernatant into a clean microcentrifuge tube and keep supernatant as **Cytoplasmic Fraction** at -80°C.

4b. (Optional): Add 250ul ice cold Solution **A** (before use, add 1ul Protease inhibitor and 5ul Phosphatase inhibitor, 1ul DTT, 10ul PMSF into 1ml Solution A) into precipitates from step **4**. Wash and centrifuge to remove residual Cytoplasmic Protein.

5. Before use, add **1ul** Protease inhibitor, **5ul** Phosphatase inhibitor, **1ul** DTT, and **10ul** PMSF into 1ml Solution **B**. Mix well and keep on ice.

6. Add 0.5ml of ice cold solution **B** into precipitates from step **4**. Vortex mixture vigorously for 10 seconds. Incubate at 4°C(on ice) for 20 minutes. Shake occasionally 3-5 times during incubation, then centrifuge at 12000rpm for 10 minutes. Transfer supernatant into a clean microcentrifuge tube and keep supernatant as **Nuclear Fraction** at -80°C.

6b. (Optional): Add 250ul ice cold Solution **B** (before use, add 1ul Protease inhibitor and 5ul Phosphatase inhibitor, 1ul DTT, 10ul PMSF into 1ml Solution A) into precipitates from step **6**. Wash and centrifuge to remove residual Nuclear Protein.

7. Before use, add **1ul** Protease inhibitor, **5ul** Phosphatase inhibitor, **1ul** DTT, and **10ul** PMSF into 1ml Solution **C**. Mix well and keep on ice.

8. Add 0.5ml of ice cold solution **C** into precipitates from step **6**. Vortex mixture vigorously for 10 seconds. Incubate at 4°C(on ice) for 20 minutes. Shake occasionally 3-5 times during incubation, then centrifuge at 12000rpm for 10 minutes. Transfer supernatant into a clean microcentrifuge tube and keep supernatant as **Membrane Fraction** at -80°C.

Notes:

1. All procedures, reagents and instruments must be pre-cold treated. This to maintain protein integrity and biological activity.

2. Optional: It is optional to do dialysis to remove the salt components in protein samples.