

Product information

QF 24 V4
CV1 2018

Extract-EZ E1 Total Subcellular Protein Extraction Kit

Catalog #: BSP073
Size: 50 Prep
Storage: 2 to -20°C*

*: Product will be shipped on ice. Upon receipt, store Extraction Buffers 1,2, 3,4 at 2-8°C. Keep Protease inhibitor and Extraction Reagent powder at -20°C. Components will have a half-year shelf life. If kept at -20°C dissolved in ddH₂O, the Extraction Reagent has a 3 month shelf-life.

Product Description:

The kit is designed for extraction of four subcellular protein fractions (Cytosol, nucleus, membrane/organelle, and cytoskeleton protein) from adherent and suspension cultured mammalian cells. The procedure is fast and simple. The whole process takes approximately 2 hours. Except for cytoskeleton protein fraction, the other three protein fractions obtained are suitable for many downstream applications such as 1-D or 2-D gel, enzyme activity assays, gel shift assay, and Western blotting. The kit is sufficient for 50X10⁷ cells adherent and suspension cultured mammalian cells.

Contents:

Description	Size
ExtractionBuffer 1	25ml
Extraction Buffer 2	25ml
Extraction Buffer 3	13ml
ExtractionBuffer 4	13ml
Protease Inhibitor	1ml
Extraction Reagent	3.8mg
Wash Buffer	100ml

Note:

Before use, add 250µl ddH₂O into Extraction Reagent. Vortex and keep at 4°C. One can also scale down volume accordingly depending on usage.

Procedure:

- For suspension cells, harvest cells by centrifugation at 700 x g for 5 minutes. Wash cells with pre-cold Wash Buffer two times, collect cells at 700 x g for 5 minutes and weigh cells. For adherent cells, discard cell media, and then wash with pre-cold Wash Buffer two times, then discard supernatants.
- Cytosolic Protein Extraction**
 - For suspension cells, add 5µl pre-cold Extraction Buffer 1, 0.05µl Extraction Reagent solution and 0.05µl Protease Inhibitor per 1mg wet cells, vortex and suspend cells. For adherent cells, add 1ml Extraction Buffer 1, 10µl Extraction Reagent solution and 10µl Protease Inhibitor into a 25T bottle, vortex.
 - Keep mixture on ice and gently agitate (at platform mixer) for 5-10 minutes until 95-100% of cells have been broken. Check efficiency of cell fractions with microscope.

C) For suspension cells, centrifuge at 480 x g for 5-10 minutes at 4 °C and store the supernatants at -70 °C. For adherent cells, incline the bottle, transfer supernatant to a clean tube. Store supernatant at -70 °C. Supernatant contains cytosolic protein. Keep pellet for membrane and organelle protein and proceed with step 3.

3. Membrane Protein and Organelle Protein Extraction

- A) For 1mg wet cell, add 5µl pre-cold Extraction Buffer 2 and 0.05µl Protease Inhibitor into the precipitates in step 2 C. Vortex vigorously to dissolve the pellet. For adherent cells, add 1ml Extraction Buffer 2, 10µl Protease Inhibitor into the 25T bottle treated in step 2C. Vortex vigorously to dissolve the pellet.
- B) Keep mixture on ice and gently agitate (at platform mixer) for 30 minutes.
- C) For suspension cells, centrifuge at 4800 x g for 10 minutes at 4 °C and collect supernatants. For adherent cells, incline the bottle and aspirate out the supernatant. Store supernatant at -70 °C. Supernatant contains membrane protein and organelle. Keep pellet for nucleoprotein extraction and proceed with step 4.

4. Nucleoprotein Extraction

- A) For 1mg wet cell, add 2.5µl pre-cold Extraction Buffer 3 and 0.025µl Protease Inhibitor into the precipitates in step 3C. Vortex vigorously to dissolve the pellet. Then homogenize with a glass homogenizer 5 times.
- B) For adherent cells, add 0.5ml Extraction Buffer 3, 5µl Protease Inhibitor into the precipitates in step 3 C. Vortex vigorously. Keep on ice and gently agitate (on a platform mixer) for 30 minutes.
- C) For suspension cells, centrifuge at 6780 x g for 5-10 minutes at 4 °C and store the supernatants at -70 °C. For adherent cells, incline the bottle and aspirate out the supernatants, then store at -70 °C. The supernatants contain nucleoprotein. Keep pellet for cytoskeleton protein extraction and proceed with step 5.

5. Cytoskeleton Protein Extraction

- A) For 1mg wet cell, add 5µl pre-cold Wash Buffer and 0.5µl Protease Inhibitor into the precipitates from step 4 C. Vortex vigorously to dissolve the pellet. Then homogenize with a homogenizer at 3000r for 5 times, then centrifuge at 13600 x g for 15 minutes at 4°C. Discard supernatants, then wash the precipitates with pre-cold acetone two times, dry the precipitates at speed vac at low temperature and keep them at -70°C.
- B) For adherent cells, add 1ml pre-cold Wash Buffer 3, 10µl Protease Inhibitor into precipitates from step 4 C. Vortex, and centrifuge at 13600 x g for 15 minutes at 4 °C. Discard supernatants, add 0.5ml Extraction Buffer 4 into precipitates, pipette them up and down until the solution is not sticky, then keep them at -70 °C.

Additional Notes:

1. Temperatures have an important effect on protein activity. The Extraction Buffer and sample must be kept at 0-4 °C and all of the operations must be carried out at low temperature.
2. To quantitate extracted protein, SK3051 or SK3061BCA quantitation kit can be used. These kits are compatible with detergent components in Extraction Buffer.
3. If the sample dissolved in Extraction buffer 4 and Extraction buffer 3 is used for 2D electrophoresis, please wash the sample dissolved in Extraction Buffer 4 and Extraction Buffer 3 with pre-cold TCA/acetone 2-3 times, then wash with ethanol and ethylether mixture (1/1 v/v). Freeze-dry the precipitates, then dissolve them in suitable 2D sample buffer. The other sample dissolved in Extraction buffer 1 and 2 can be used directly in 2D sample buffer.
4. In step 2 and 3, when vortexing suspended precipitates, do not vibrate drastically, only rotate the bottle at level, in case organelles and cell nuclei break. This has an important effect on the separation of different subcellular proteins.
5. The kit can only be used for in vitro experiments.



PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY.
NOT INTENDED FOR HUMAN OR ANIMAL USE.