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Product information

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Cytosol & Mitochondria Protein Extraction Kit

Catalog #: BSP051
Size: 50 preps
Storage: Mixed components storage*

*: Product shipped with ice pack. Check storage conditions.

Product Description:

Designed to facilitate the study of apoptotic and signal transduction pathways, the kit provides unique formulations of buffers and reagents that enable isolation of highly enriched mitochondrial and cytosolic fractions from various mammalian cell lines, including both apoptotic and non-apoptotic cells. The fractionation procedure is simple and easy to perform. No ultracentrifugations are required and no toxic chemicals are involved. The enriched fractions can be used to study translocation of a variety of proteins between the mitochondrial and cytosolic fractions by Western blot analysis, ELISA, 2D electrophoresis or other assays. After lysis, different cellular compartments are separated by centrifugation of the cellular lysate at different speeds to isolate, successively, the nuclei, mitochondria and cytosol. The kit is sufficient for 50X10^7 cells or 50x200 mg of tissue samples.

Storage:

Upon receipt, store Cytosolic Buffer at 2-8°C. Keep Protease inhibitor, Mitochondria Buffer and Phosphatase inhibitor, DTT solution at -20°C.

Composition:

Cytosolic Buffer	55 ml
Mitochondria Buffer	5 ml
Protease Inhibitor	60 µl
Phosphatase Inhibitor	300 µl
DTT (1M)	60 µl

Procedure:

1. Wash the cells with ice-cold PBS three times from cell suspension culture. Add ice-cold PBS and scrape the cells off the dish using a cell scraper. Transfer the cells to a pre-chilled centrifuge tube and spin at 600 g for 5 minutes at 4°C. Remove supernatant and wash cell pellet by gently resuspending it ice-cold PBS. Spin at 600 g for 5 minutes at 4°C and remove supernatant. One extraction requires ~1x10^7 cells.

NOTE: For tissue, one extraction requires ~200 mg of tissue sample. Remove fat and nerve tissue at best, cut it into small pieces, and then wash them with pre-cold PBS for three times.

2. Add 1 ml ice cold Cytosolic Buffer (before use, add 1 µl Protease inhibitor and 5µl Phosphatase inhibitor, 1 µl DTT into 1 ml Cytosolic Buffer). Resuspend the cell pellet by pipetting up and down gently with a pipette. Incubate on ice for 15 minutes, and then transfer to a pre-chilled pestle homogenizer.

3. On ice, homogenize the cells with 30-50 strokes using a homogenizer. Transfer the supernatant to a pre-chilled microcentrifuge tube and vortex. Check the efficiency of cell fracture and ensure more than percent 90 cells have been broken.

4. Transfer the above homogenized solution into a new pre-cold 1.5 ml centrifuge tube, and then centrifuge at 600 g at 4°C for 10 minutes. The supernatant contains cytosol portion, including the mitochondria.

NOTE: The resulting pellet is the nuclei, cellular debris and intact cells.

5. Transfer the supernatant to a fresh, pre-chilled microcentrifuge tube; spin the supernatant at 600 g for 10 minutes at 4°C to remove any residual nuclei and cellular debris.

6. Transfer the supernatant to a fresh, pre-chilled microcentrifuge tube; spin the supernatant at 10,000 g for 30 minutes at 4°C to pellet the mitochondria. Spin the supernatant at 18,000 g for at least 20 minutes at 4°C to remove tiny residual mitochondria. Transfer the resulting supernatant to a fresh, pre-chilled microcentrifuge tube. This is the **Cytosolic Fraction**. Aliquot and keep at –80°C.
7. Wash the mitochondrial pellet with 100 µl Cytosolic Buffer (before use, add 1 µl Protease inhibitor and 5 µl Phosphatase inhibitor, 1 µl DTT into 1 ml Cytosolic Buffer) and spin at 10,000 g for 10 minutes at 4°C. Remove and discard the supernatant.
8. Lyse the mitochondrial pellet by adding 100 µl Mitochondria Buffer (before use, add 1 µl Protease inhibitor and 5 µl Phosphatase inhibitor, 1 µl DTT into 1 ml Mitochondria Buffer) and incubating on ice for 30 minutes. After incubation, vortex for 10 seconds to mix thoroughly. Spin at 10,000 g for 10 minutes at 4°C. The supernatant is the **denatured Mitochondrial protein fraction** for 2D electrophoresis.

   NOTE: To keep intact Mitochondria with no lysis, resuspend mitochondrial pellet in Cytosolic Buffer and keep at 2-8 °C.

   NOTE: For native Mitochondrial protein, please lyse the mitochondrial pellet by adding 100 µl native Mitochondria Buffer (20mMTris-HCl pH 7.5, 2mM EGTA, 2mM EDTA, 1% Tritonx-100, before use, add 1 µl Protease inhibitor and 5 µl Phosphatase inhibitor, 1 µl DTT into 1 ml native Mitochondria Buffer), incubating on ice for 100 minutes. After incubation, vortex for 30 seconds to mix thoroughly. Spin at 10,000 g for 10 minutes at 4°C and the supernatant is the native Mitochondrial protein Fraction..
9. Measure the protein concentration of each fraction by Non-Interfering Protein Concentration Determination Kit (SK3071).
10. Aliquot the fractions and store at -80°C. Avoid freeze/thaw cycles.

Additional Notes:

1. All reagents and instruments must be pre-cold to maintain protein activity and integrity.
2. For Cytosol and Mitochondria Protein quantitation, Non-Interfering Protein Concentration Determination Kit (Sk3071) may be used.
3. The number of strokes required to lyse the cells varies on the cell line. For HeLa, PC3, DU145, JCA1 and ALVA31 cell lines, between 30 and 50 strokes seem to work well. In contrast, mitochondria from Jurkat and LNCaP cells appear to be more fragile and only 30 or fewer strokes should be applied. You may need to optimize the lysis procedure for your given cell line and/or treatment.
4. For apoptosis-induced cells, detachment of dead cells during the wash step will reduce the number of cells (relative to a plate of “normal” cells) that are obtained by scraping the plate. Therefore, to obtain fractions of an adequate concentration, buffer volumes may need to be reduced to coincide with the reduced number of cells being fractionated.
5. Lysis is performed in a pestle homogenizer using the gentle Cytosolic Buffer. This helps to eliminate accidental rupture of the mitochondria and the subsequent leakage of mitochondrial proteins (such as cytochrome c) into the cytosolic fraction.
6. If Mitochondrial protein leakage is observed, cell lysis may be too vigorous, please reduce the number of strokes with the homogenizer to 30 strokes.
7. If low protein concentration in cytosolic fraction, cell lysis may not efficient, please increase the number of strokes performed with the homogenizer.
8. When store the Mitochondria Buffer at -20°C, maybe appear some insoluble precipitates, please heat the buffer to 37°C about 5 minutes or so for completely dissolving precipitates.