Minute™ Cytosolic and Nuclear Extraction Kit for Frozen/Fresh Tissues

Cat. No. NT-032

Description

Preparation of cytosolic and nuclear fractions from cultured cells and tissues is a common practice. Cell fractionation from cultured cells is relatively easy and straight forward. However, a clear separation of cytosolic and nuclear fractions from tissues especially frozen tissues is challenging. The cross-contaminations from frozen tissues is especially troublesome due to the altered tissue cellular structure caused by freeze and thaw cycle, improper homogenization process and inadequate extraction buffers used. Majority of current commercial kits are designed to treat cultured cells and tissues similarly without paying sufficient attention to the structure difference between the two types of samples. As a result, cross-contamination of cytosolic and nuclear fractions from fresh/ frozen tissues remain a major problem (see references 1 and 2 below). This novel cytosolic and nuclear extraction kit is specially designed to address the issue by employing proprietary buffers and unique protocol to minimize the cross-contaminations.

Applications

The kit is designed to rapidly extract cytoplasmic and nuclear proteins from tissue samples for applications such as SDS-PAGE, immunoblotting, ELISA, IP, protein localization, gel mobility shift assays, 2-D gels and other applications. The whole protocol can be done in 30 min.

Kit Components

1. Buffer A 25 ml
2. Buffer B 10 ml
3. Buffer D 1.5 ml
4. Buffer N 1.5 ml
5. 1.5 ml microfuge tube X 40
6. Pestle for 1.5 ml tube X 2
7. Protein Extraction Powder X 2g

Shipping: Ambient temperature **Storage:** Store the kit at RT

Important Product Information

1. Chill buffer A and buffer B on ice prior to use.
2. Perform all centrifugation at 4^{o}C or in a refrigerated centrifuge
3. It is recommended to add proteinase inhibitor cocktail to aliquoted buffer A prior to use

Invent Biotechnologies, Inc., 1864 Berkshire Ln, N, Plymouth MN 55441
Tel: (866)-817-7611, Fax: 952-400-6889, www.inventbiotech.com

4. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to aliquots of buffer A and B.
5. BCA kit (Pierce) is recommended for protein concentration assay
6. White precipitate may be seen in buffer D when room temperature is below 20°C. Heat the buffer at 37°C water bath to dissolve the precipitate prior to use.

Protocol

1. Place 20-30 mg fresh/frozen tissue in a 1.5 ml tube provided in the kit, add 250 µl buffer A to cover the tissue and incubate on ice for 5 min. Gently grind the tissue with the pestle provided using back and forth twisting force for about 1 min (40-60 times) The pestle is reusable, for cleaning, rinse it with tape water and dry with paper towel.

2. Centrifuge at 14,000 X g for 5 min. Transfer 200 µl supernatant (this is cytosolic fraction) to a fresh microfuge tube from your lab. (if the supernatant is not clear enough, centrifuged at 14,000 X g for another 5 min to further clarify). Grind the wet pellet with the pestle as described above (grind 60-100 times). While the pestle is still in the tube, add 0.8 ml buffer A to the tube. Grind a few more times and remove the pestle. Mix well by pipetting the homogenate up and down for 15-20 time with a 1 ml pipette tip. Incubate the tube on ice for 5-8 min to allow larger tissue debris sink to the bottom by gravity Transfer 0.7 ml supernatant to a fresh 1.5 ml tube provided in the kit (try to avoid larger tissue debris at the bottom of the tube).

3. Centrifuge at 500 X g for 2 min. remove and discard the supernatant. Resuspend the pellet in 0.5 ml buffer B by vortexing 10-20 seconds. Incubate on ice for 5 min. Centrifuge at 2000 X g for 2 min. Remove the supernatant completely.

4. The pellet contains isolated nuclei. There are two options for extracting nuclear proteins for different downstream applications:

   A. **Denatured nuclear protein extraction**: Resuspend the pellet in 50 µl buffer D (the suspension may be viscous, this is normal). Weight out 50-60 mg protein extraction powder and add to the pellet (try your best to avoid touching the wall of the tube). Grind with the pestle with back and forth twisting force for about 60-100 times. While the pestle is still in the tube, add 50 -150ul buffer A to the tube and grind for a few more times (the exact amount of buffer A used depends upon the size of the pellet. the pestle is reusable, for cleaning rinse with tape water and dry with paper towel). Centrifuge the tube at 14,000 X g for 5 min. Transfer the supernatant to a fresh tube from your lab (this is nuclear fraction). Proteinase inhibitor cocktails can be added to the nuclear extract and store at-20-80°C. The nuclear extract is suitable for SDS-PAGE, Western blotting and other applications. The protein concentration is typically 1-2mg/ml.

Invent Biotechnologies, Inc., 1864 Berkshire Ln, N, Plymouth MN 55441
Tel: (866)-817-7611, Fax: 952-400-6889, www.inventbiotech.com

B. **Non-denatured nuclear protein extraction**. Resuspend the pellet in 50 µl buffer N. Incubate at ambient temperature for 5 min. Weight out 50-60 mg protein extraction powder and add to the pellet (try your best to avoid touching the wall of the tube). Grind with the pestle provided with back and forth twisting force for about 60-100 times. While the pestle is still in the tube, add 50 -150 µl buffer A to the tube and grind for a few more times (the exact amount of buffer A used depends upon the size of the pellet). Centrifuge the tube at 10,000 X g for 5 min. Transfer the supernatant to a fresh tube from your lab (this is nuclear fraction). Proteinase inhibitor cocktails can be added to the protein extract and store at-20-80C. The nuclear extract is suitable for ELISA, IP, enzymatic activity assay, gel retardation assay and other applications. The protein concentration is typically 0.5-1mg/ml.

About cytosolic and nuclear markers

Many antibodies have been used in Western blotting for checking the cross-contamination of isolated fractions. Commonly used cytosolic markers found in literatures include but not limited to GAPDH, HSP90, LC3 A/B, Tubulin, HIF1, ESR1, ACTB and Actin etc. We recommend GAPDH, HSP90 or LC3 A/B and not recommend beta-actin because actin is also present in nucleus (see references 3 and 4). For nuclear markers we recommend Lamin B1, Lamin A/C, SC-35 or histones.

References

1. Kuster et al (2011) Nuclear protein extraction from frozen porcine myocardium. J Physiol. Biochem. 67:165-173.
2. Murray et al (2009) Assessment of ProteoExtract subcellular fractionation kit reveals limited and incomplete enrichment of nuclear subproteome from frozen liver and heart tissue. Proteomics. 9(15):3934-3938.
3. Dopie et al (2012) Active maintenance of nuclear actin by importin 9 supports transcription. PNAS E544-E552.
4. Falahzadeh et al (2015) The potential roles of actin in nucleus. Cell J. 17:7-14.

Invent Biotechnologies, Inc., 1864 Berkshire Ln, N, Plymouth MN 55441
Tel: (866)-817-7611, Fax: 952-400-6889, www.inventbiotech.com