Minute™ Cytosolic Proteasome Enrichment Kit

Cat. No. PT-040

Description

The proteasome is a non-lysosome protein degradation system that requires metabolic energy and polymerization of ubiquitin. The 26S proteasome is made up of two subcomplexes (a 20S proteasome and one or two19S regulatory particles). Rapid and gentle isolation of proteasome from cells/tissues is essential for the studies of proteasome structure and function. The proteasomes have been found mainly localized in cytosol but also detected in nucleus. Traditionally, ultracentrifugation and affinity isolation are the most common methods for isolation of proteasomes. These methods, though relatively effective, are usually time consuming with low yield. Many affinity-based methods require harsh elution condition that may affect the activity of isolated proteasomes and limit certain downstream applications. To overcome these shortcomings, we have developed a spin-column based technology using a proprietary precipitation buffer for enrichment of proteasome from cytosol by first removing nuclei and majority of organelles followed by preferential precipitation of cytosolic proteasomes. The nuclear proteasomes are excluded with this kit **(Nuclear Proteasome Enrichment Kit is available under Cat # PN-041).** The protocol is simple and rapid with high yield. The gentle protocol maintains the association of proteasomes with ubiquitin and other proteins. This kit is useful for the studies of proteasome structure and function. The enriched proteasomes can also be used as a starting material for affinity purifications.

Kit Components (20T):

1.	Buffer A	10 ml
2.	Buffer B	10 ml
3.	Plastic rods	2
4.	Filter Cartridge	20
5.	Collection Tube	20

Additional Materials Required

1 X PBS, table-top micro centrifuge with a maximum speed of 14,000-16,000 X g. **The centrifuge should be able to reach maximum speed within 10 seconds.**

Shipping and Storage: Ship at ambient temperature and store at 4°C.

Important Information:

1. Read the entire procedures carefully. **All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated microfuge.**
2. **Protease inhibitor cocktails** can be added to buffer A prior to use.
3. **Phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use for protein phosphorylation studies.
4. It is recommended to use BCA Protein Assay Kit (Pierce) for determination of protein concentration.

Protocol

Note: Warm buffer B to room temperature and mix well prior to use.

1. Place the filter cartridges in a collection tube and incubate on ice. **Pre-chill buffer A on ice.**

2. **A. For cultured cells**, collect 30-40 x 10^6 cells by low speed centrifugation (500-600 X g for 5 min). Wash cells once with cold PBS. Resuspend the pellet in 450 µl buffer A. **Vortex vigorously for 10-30 seconds**. Immediately transfer the cell suspension to the filter cartridge. Go to step 3.

   B. For soft tissue samples, place 30-40 mg tissue (fresh or frozen) in a filter cartridge. Add 200 µl buffer A to the filter and grind the tissue with the plastic rod by pushing the tissue against the surface of the filter repeatedly with twisting force for 2-3 min. After grinding, add 250 µl buffer A to the same filter cartridge. Go to step 3. **For muscle tissues,** Place tissue on surface of a clean glass or plastic plate. Mince the tissue with a sharp blade into small pieces (meat slurry). Transfer the minced tissue to the filter cartridge and grind as above. **The plastic rod is reusable. Clean it with 70% alcohol or water.**

3. Cap the filter cartridge, invert a few times and centrifuge at 16,000 X g for 30 seconds. For cultured cells, resuspend the cell lysate and re-pass through the same filter one more time.
4. Remove and discard the filter, vortex briefly and centrifuge the collection tube at 16,000 X g for 30 min. Transfer 400 µl supernatant to a fresh 1.5 ml microfuge tube. The pellet contains mainly cell debris, nuclei, plasma membranes and organelles.
5. Add 400 µl buffer B to the supernatant (buffer B to supernatant =1:1). Mix by vortexing for 10-20 seconds. Incubate on ice for 10 min. Centrifuge at 10,000 X g for 10 min. Remove and discard supernatant.
6. Centrifuge the tube briefly at 10,000 X g to bring down residual reagent on the wall of the tube. Remove supernatant completely. The pellet contains highly enriched proteasomes that is readily dissolved in aqueous solutions.
7. Resuspend the pellet in 100-200 µl PBS or other buffer for downstream applications. The protein yield is typically 1-2 mg/ml and proteasome activity is retained. The resuspended pellet contains salts that may interfere with 2D gel analysis. The salts can be removed by dialysis or passing through a desalting column.