Minute™ Nuclear Proteasome Enrichment Kit

Cat. No. PN-041

Description

The proteasome is a protein degradation system that requires metabolic energy and polymerization of ubiquitin that is different from lysosome-based protein degradation. The 26S proteasome is made up of two subcomplexes (a 20S proteasome and one or two19S regulatory particles). The proteasomes have been found mainly localized in the cytosol but also detected in the nucleus. Traditionally, ultracentrifugation and affinity isolation are the most common methods for the isolation of proteasomes. These methods, though relatively effective, are usually time-consuming with low yield. Many affinity-based methods require harsh elution conditions that may affect the activity of isolated proteasomes and limit certain downstream applications. In most cases, the methods can't distinguish proteasomes derived from cytosol or nucleus. To overcome these shortcomings, we have developed spin-column-based kits for the enrichment of proteasome from nuclei (**Cytosolic Proteasome Enrichment Kit is also available under Cat # PT-040**). The protocol is simple and rapid with a high yield. The gentle protocol maintains the association of proteasomes with ubiquitin and other proteins and is useful for the studies of proteasome structure and function. The highly enriched proteasomes can also be used as a starting material for affinity purifications.

Kit Components (20T):

1.	Buffer A	30 ml
2.	Buffer B	8 ml
3.	Pestle for 1.5 ml tube	2
4.	Filter cartridge with collection tube	40
5.	Protein extraction powder	2.0 g

Additional Materials Required

Vortexer, and table-top micro centrifuge with a maximum speed of > 16,000 X g**.**

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 microcentrifuge.**
2. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. Protease inhibitor cocktails can be added to aliquot of buffer A and buffer B prior to use.

Protocol

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

1. Place the filter cartridges in collection tubes 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 500 µl buffer A and incubate on ice for 5 min. Transfer the cell suspension to the filter cartridge. Go to step 3.

   B. For soft tissue samples. Place 50-60 mg tissue (Frozen or fresh) in a filter cartridge. Add 200 µl buffer A to the filter and grind the tissue using the flat end of the pestle provided in the kit by pushing the tissue against the surface of the filter repeatedly with twisting force for 2-3 min. After grinding, add 300 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 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, Cap the tube and vortex briefly. Centrifuge the tube at 600 X g for 5 min. Remove and discard supernatant.
5. Resuspend the pellet in 0.4 ml buffer A and transfer to a 1.5 ml tube. Centrifuge at 800 X g for 3 min. Remove supernatant completely and add 50 µl buffer A and 60-80 mg protein extraction powder to the pellet.
6. Grind the pellet with the plastic pestle for 2-3 min with twisting force (longer grinding can increase final yield). Add 400 µl buffer A to the tube. Resuspend by pipetting up and down for a few times with 1 ml pipette tip and transfer the nuclear lysate to a fresh filter cartridge with collection tube.
7. Cap the filter and centrifuge the tube at 16,000 X g for 15 min. Transfer 400 µl supernatant to a fresh 1.5 ml microfuge tube. The supernatant is extracted native total nuclear matrix protein. If desired it can be used as a starting material for affinity-based proteasome purification,
8. Add 400 ul buffer B to the supernatant (the buffer B to supernatant ratio = 1:1) mix well by vortexing briefly. Incubate on ice for 10 min.
9. Centrifuge at 16,000 X g for 10 min. Pour out the supernatant. The pellet is enriched nuclear proteasomes. Centrifuge the tube at 16,000 X g for 2 min to bring down residual reagent and remove it completely. Resuspend the pellet in 50-150 µl PBS or other buffer of your choice for downstream applications. In many cases, precipitated proteasomes may attach to the wall of the tube. Be sure to resuspend it completely by pipetting up and down along the wall. 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.