Minute™ Lysosome Isolation Kit

(for mammalian cells/tissues)

Cat. No. LY-034

Description

Lysosomes are spherical vesicles in eukaryotic cells, which are responsible for waste removal. The digestive enzymes contained in lysosomes play a vital role in digesting excess or worn out organelles, food particles and engulfed viruses and bacteria. Lysosomes are relatively large organelles ranging in size from 0.1 to 1.2 um. Ability to isolate lysosomes is an important first step for the studies of autophagy, protein degradation and protein recycling in a cell. Traditional methods for isolating lysosomes are based on density gradient ultracentrifugation. Large amount of starting material is required and the methods are tedious and time consuming with significant cross-contamination. Currently, all commercial kits for lysosome isolation are based on the methods developed in 1970s of last century. Unlike any other lysosome isolation kit in the market, our kit employees a patented spin-column based technology that is simple, rapid and efficient. The amount of starting cells/tissues required is much smaller than that of traditional methods. This kit can significantly enrich lysosomes from cultured cells or tissues without using a Dounce homogenizer and ultracentrifugation. The whole protocol can be done in less than 1.5h.

Kit Components (20 prep)

1.	Buffer A	15 ml
2.	Buffer B	2 ml
3.	Plastic rods	2
4.	Filter Cartridge	20
5.	Collection Tube	20

Additional Materials Required

1 X PBS, Vortexer, Table-Top Micro centrifuge with maximum speed of 16,000 Xg. **The centrifuge should be able to reach maximum speed within 10 seconds.**

Shipping and Storage: Ship at ambient temperature and store the kit at 4^{o}C

Important Information:

1. Read the entire procedures carefully. Chill protein extraction filter cartridge with collection tube on ice prior to use.
2. **All centrifugation steps should be performed at 4^{o}C in a cold room or in a refrigerated microfuge.**

3. To study protein phosphorylation, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. If protein degradation is a concern, add protease inhibitor cocktails to buffer A and B prior to use.
4. It is recommended to use BCA Protein Assay Kit for determination of protein concentration (Pierce, Cat #:23227).

Protocol

1. Place the filter cartridges in a collection tube and incubate on ice.

2. **For cultured cells**, collect 25-30 X 10^6 cells by low speed centrifugation (500-600 X g for 5 min). Go to step 3a. **For tissue samples**, go to step 3b.

3a. Wash cells once with cold PBS. Remove supernatant completely and resuspend the pellet in 500 µl buffer A. Incubate the cell suspension on ice for 5-10 min. **Vortex the tube vigorously for 10-30 seconds**. Immediately transfer the cell suspension to the filter cartridge. Go to step 4.

3b **For tissue samples**, place 20-30 mg tissue (fresh or frozen) in a filter cartridge. Add 200 µl buffer A to the filter and grind the tissue with a plastic rod for one min by pushing the tissue against the surface of the filter repeatedly with twisting force. After grind, add 300 µl buffer A to the same filter cartridge, mix by pipette up and down a few times and incubate the tube on ice with **cap open** for 5 min. Go to step 4. **The plastic rod is reusable. Clean it with 70% alcohol or water.**

4. Cap the filter cartridge, invert a few times and centrifuge at 16,000 X g for 30 seconds. (optional: the flow through in the collection tube can be resuspended and re-pass through the same filter again. This may increase the yield).

5. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds. Centrifuge at 2000 X g for 3 min (the pellet contains nuclei, large cell debris and some un-ruptured cells).

6. Transfer all supernatant to a fresh 1.5 ml microfuge tube and centrifuged at 4°C for 15 min at 11,000 X g. The pellet contains mainly mitochondria and cell debris.
After centrifugation, carefully transfer 400 µl supernatant to a fresh 1.5 ml tube and spin at 16,000 X g at 4°C for 30 min. Remove the supernatant completely.

7. Resuspend the pellet in 200 µl cold buffer A by repeat pipetting up and down for 60-100 times and vortex vigorously for 20 seconds. Centrifuge at 2000 X g for 4 min. Carefully transfer the supernatant to a fresh 1.5 ml tube. Add 100 µl buffer B to the tube and vortex briefly to mix well (the supernatant to buffer B ratio is 2:1). Incubate the tube on ice for 30 min and centrifuge at 11,000 X g for 10 min. Remove the supernatant completely. Spin the tube at 11,000 X g for a few seconds to bring down residual reagent and remove it completely.

8. Resuspend the pellet in 50-150 ul PBS or other buffers. This is highly enriched lysosome fraction. The lysosome yield is typically 50-100 µg/sample. If the pellet protein concentration is too low, increase the starting material. The insoluble lysosome fraction can be dissolved in following reagents depending upon downstream applications.

Following protein solubilization reagents are recommended.

Product Name	Cat. No.	Applications
Minute™ Denaturing Protein Solubilization Reagent	WA-009	SDS-PAGE electrophoresis and Western blotting, trypsin digestion, purification of proteins with biotin labeling or histidine labeling, etc.
Minute™ Non-Denatured Protein Solubilization Reagent	WA-010	ELISA, immunoprecipitation/Co-IP, enzymatic activity determination and other applications.
Minute™ Protein Solubilization Reagent for MS	WA-011	Trypsin digestion and subsequent mass spectrometry analysis.