Minute™ Lysosome Isolation Kit
(For mammalian cells/tissues)

Cat. No. LY-034

Description

Lysosomes are spherical vesicles in eukaryotic cells responsible for waste removal. The digestive enzymes 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. The ability to isolate lysosomes is an essential first step in studying autophagy, protein degradation, and recycling in a cell. Traditional methods for isolating lysosomes are based on techniques developed in the 1970s, requiring a large amount of starting material and a tedious process. Significant cross-contaminations are inevitable. This kit employs a patented spin-column-based technology that is simple, rapid, and efficient. Native lysosomes can be significantly enriched without using a Dounce homogenizer and ultracentrifugation. The protocol takes less than 90 minutes, using only 20-30mg of the starting material.

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 Microcentrifuge with a maximum speed of 16,000 X g. **The centrifuge should be able to reach top speed within 10 seconds.**

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

Important Information:

1. Read the entire protocol carefully. Chill protein extraction filter cartridge with collection tube on ice before use.
2. **All centrifugation steps should be performed at 4°C in a cold room or in a refrigerated microfuge.**
3. For protein phosphorylation studies, **phosphatase inhibitors** (such as PhosStop from Roche) should be added to buffer A prior to use. Add protease inhibitor cocktails to buffer A and B If protein degradation is a concern.

4. BCA Protein Assay Kit (Pierce, Cat #:23227) is recommended to determine protein concentration.

Protocol

1. Place the filter cartridges in the collection tubes and incubate them on ice.

2. **For cultured cells**, collect 25-30 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 5-10 min. **Vortex the tube vigorously for 10-30 seconds**, and immediately transfer to the filter cartridge. Go to step 4.

2. **For tissue samples**, place 20-30 mg of 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 1 min by pushing the tissue against the surface of the filter repeatedly with twisting force. After grinding, add 300 µl buffer A to the same filter cartridge, pipette up and down to mix, and incubate on ice **with cap open** for 5 min. Go to step 4.
*****The plastic rod is reusable. Clean 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-passed through the same filter. This may increase the yield).

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

6. Transfer all supernatant to a fresh 1.5 ml microfuge tube and centrifuge at 4ºC 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 4ºC for 30 min. Remove the supernatant completely.

7. Resuspend the pellet in 200 µl cold buffer A by pipetting up and down 60-100 times and vortex vigorously for 20 seconds. Centrifuge at 2000 X g for 4 min. Transfer the supernatant to a fresh 1.5 ml tube, add 100 µl buffer B, 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 all supernatant, and spin the tube at 11,000 X g briefly to bring down residual buffer; remove it altogether.

8. Resuspend the pellet in 50-150 µl PBS or buffer of your choice. This is the highly enriched lysosome fraction.

Tech Notes:

The Typical protein yield is 50-100 µg/sample. Increase the starting material if the target protein concentration is low. If there is no visible pellet in step 8, check the supernatant and pellet in step

6 for the presence of lysosomes after 11,000 X g spin. If the majority of lysosomes are found in the pellet, reduce the centrifugal force from 11,000 X g to 8,000-10,000 X g in step 6.

Depending upon downstream applications, the insoluble lysosome fraction can be dissolved in the following reagents.

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.