Minute™ Lysosome Isolation Kit

For mammalian cells/tissues

Cat. No. LY-034

Description

Lysosomes, found in eukaryotic cells, are spherical vesicles responsible for the removal of cellular waste. These organelles contain digestive enzymes crucial for breaking down excess or worn-out organelles, food particles, as well as viruses and bacteria that have been engulfed. Lysosomes exhibit a relatively large size, ranging from 0.1 to 1.2 micrometers. In the realm of cell research, the initial isolation of lysosomes is a pivotal step when investigating processes like autophagy, protein degradation, and recycling.

Traditional methods for lysosome isolation, which originated in the 1970s, are associated with challenges such as the need for substantial starting material and a time-consuming procedure that often leads to significant cross-contaminations. However, a groundbreaking solution is offered by this kit, utilizing patented spin-column-based technology that is characterized by its simplicity, speed, and efficiency. With this kit, native lysosomes can be notably enriched without the requirement of a Dounce homogenizer or ultracentrifugation. The entire protocol can be completed in less than 90 minutes, utilizing only 20-30 milligrams of the starting material.

Kit Components (20 preps)

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 4ºC

Important Information:

1. Please carefully review the entire protocol. Prior to use, ensure the filter cartridge with the collection tube is chilled on ice.
2. Perform all centrifugation steps at 4°C, either in a cold room or using a refrigerated microfuge.
3. If you are conducting protein phosphorylation studies, it's essential to add phosphatase inhibitors (e.g., PhosStop from Roche) to buffer A before usage. Additionally, include protease inhibitor cocktails in buffer A and B if you are concerned about protein degradation.
4. We recommend using the BCA Protein Assay Kit to determine protein quantification.

5. Please note that the yield and purity of isolated lysosomes may vary depending on the specific cell/tissue types and the amount of starting material used. You may need to optimize the protocol to achieve the best results (see technical notes below).

Protocol

1. Take the filter cartridges and place them inside the collection tubes. Allow them to incubate on ice.
2. **For cultured cells:** Collect 25-30 X 10^6 cells by low-speed centrifugation at 500-600 X g for 5 minutes. Wash the cells once with cold PBS, then resuspend the pellet in 500 µl of buffer A. Incubate on ice for 5-10 minutes. Next, vigorously vortex the tube for 10-30 seconds and immediately transfer it to the filter cartridge.
   For tissue samples: Place 20-30 mg of tissue (either fresh or frozen) in a filter cartridge. Add 200 µl of buffer A to the filter and grind the tissue with a plastic rod for 1 minute, repeatedly pushing the tissue against the filter surface with a twisting force. After grinding, add 300 µl of buffer A to the same filter cartridge, pipette up and down to mix, and incubate on ice with the cap open for 5 minutes.
   (Note: The plastic rod is reusable; clean it with 70% alcohol or water.)
3. Cap the filter cartridge, invert it a few times, and centrifuge at 16,000 X g for 30 seconds. (Optional: you can resuspend the flow-through in the collection tube and re-pass it through the same filter; this may increase the yield).
4. Discard the filter and resuspend the pellet by vigorously vortexing for 10 seconds. Centrifuge at 2,000 X g for 3 minutes (the pellet contains nuclei, large cell debris, and un-ruptured cells).
5. Transfer all the supernatant to a fresh 1.5 ml microfuge tube and centrifuge at 4°C for 15 minutes at 11,000 X g. The pellet mainly contains mitochondria and cell debris. After centrifugation, carefully transfer 400 µl of the supernatant to a fresh 1.5 ml tube and spin at 16,000 X g at 4°C for 30 minutes. Remove the supernatant completely.
6. Resuspend the pellet in 200 µl of cold buffer A by pipetting up and down 60-100 times and vortex vigorously for 20 seconds. Centrifuge at 2,000 X g for 4 minutes. Transfer the supernatant to a fresh 1.5 ml tube, add 100 µl of buffer B, and briefly vortex to mix well (the supernatant to buffer B ratio is 2:1). Incubate the tube on ice for 30 minutes and centrifuge at 11,000 X g for 10 minutes. Remove all the supernatant, and briefly spin the tube at 11,000 X g to remove any residual buffer.
7. Resuspend the pellet in 50-150 µl of a detergent-containing buffer for downstream analysis.

Tech Notes:

1. The typical protein yield per sample ranges from 50-100 µg. If the yield is lower than expected, consider increasing the amount of starting material. In case you do not observe a visible pellet in step 6 after the 11,000 X g spin, examine the supernatant and pellet from step 5 (following the 11,000 X g spin) for the presence of lysosomes. If you find that the majority of lysosomes are located in the pellet, reduce the centrifugal force from 11,000 X g to 8,000-10,000 X g.
2. The isolated lysosome pellet is water-insoluble and must be dissolved in a detergent-containing buffer for protein quantification. If the pellet doesn't solubilize efficiently using WA-009 (see table below), add SDS to WA-009 to reach a final concentration of 0.4% and increase the volume of the protein solubilization reagent. Some components in buffer B might interfere with mass spectrometry analysis and should be removed after trypsin digestion.
3. To assess the yield and purity of the isolated lysosomes, we recommend comparing them to the total cell/tissue lysate in Western blotting (WB) using an antibody specific to lysosome markers like Lamp1/Lamp2. It's crucial to ensure equal protein loading in SDS-PAGE. Staining the post-transfer blot with Ponceau Red can provide insights into significant variations in protein loading.

4. The extent of lysosome enrichment varies depending on the sample type. It's known that intracellular membranous structures are interconnected, and certain cytosolic marker proteins, such as actin and tubulin, may also associate with organelles. Therefore, detecting these proteins in the isolated lysosome fraction is not unexpected.
5. The yield of lysosomes depends primarily on two factors: A. the amount of starting material and B. the efficiency of cell membrane rupture. You can easily evaluate cell membrane rupture efficiency by staining cells with trypan blue before and after passing them through the filter. Cell viability should be over 90% before and less than 30% after passing cells through the filter. If cell rupture efficiency is low, a solution is to resuspend cells in buffer A and freeze and thaw at -80°C twice, and then follow the standard protocol.
6. Buffer B contains PEG that may interfere with mass spectrometry and needs to be removed prior to the analysis (reference: Zhao C, O'Connor PB. ***(Removal of polyethylene glycols from protein samples using titanium dioxide. Anal Biochem. 2007 Jun 15;365(2):283-5).***
7. Depending on your intended downstream applications, you can dissolve the insoluble lysosome fraction in the following recommended reagents:

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.