Lysosome Isolation Kit

Cat. No. LYS-007K **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Lysosome Isolation Kit provides a procedure for isolating enriched or purified lysosomal fraction from animal tissues and cultured cells by differential centrifugation followed by density gradient centrifugation.
Applications	Isolation of intact functional lysosomes from tissues and cultured cells Lysosome studies like enzyme activity or uptake studies Protein profiling of Lysosomes by SDS-PAGE and Western blot
Storage	Store kit at -20centigrade, protected from light. Thaw before use. Read the entire protocol before performing the assay.
Kit Components	• Lysosome Isolation Buffer 25 ml • Lysosome Enrichment Buffer 100 ml • Lysosome Gradient 85 ml • Protease Inhibitor Cocktail 1 ml
Materials Required but Not Supplied	Bench-top centrifuge with variable speed and controlled low-temperature capabilities Ultracentrifuge, rotor, and compatible tubes Glass Dounce Homogenizer PBS
Features & Benefits	• Simple & convenient protocol • The kit provides unique formulations of ready-to-use buffers and reagents to isolate lysosomes with minimal sample preparation

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Preparation	Lysosome Isolation Buffer and Lysosome Enrichment Buffer: Determine the needed volume and add Protease Inhibitor Cocktail at a ratio of 1:1000 (1 µto 1 ml Buffer) to Lysosome Isolation Buffer and Lysosome Enrichment Buffer. Precooled Ultracentrifuge and accessories: Place rotor, tubes, reagents, and dounce homogenizer on ice/refrigerator.
Separation Protocol	1. Sample Preparation: a. Cultured Cells: Pellet 2 x10^7 cells by centrifugation at 600 x g for 10 min. Carefully remove and discard the supernatant. b. Tissues: Isolate the tissue of interest (~100 mg). Immerse the sample in 1 ml of ice-cold PBS. Rinse the tissue twice in 1 ml PBS to remove blood. Mince the tissue on ice into small pieces using scissors. Discard PBS used for mincing and replace it with 800 µof Lysosome Isolation Buffer. 2. Procedure: a. Cultured Cells: Add 500 µof Lysosome Isolation Buffer to the pellet and vortex for 5 seconds, followed by incubation on ice for 2min. Homogenize the cells using a precooled Glass Dounce Homogenizer. Stroke the sample 20-30 times on ice. Transfer the homogenate to a fresh tube. Add 500 µof Lysosome Enrichment Buffer. Invert the tube several times to mix. Centrifuge at 500 x g for 10 min at 4centigrade. Collect the supernatant in a separate tube and keep on ice. b. Tissue: Homogenize the tissue using a precooled glass homogenizer. The optimal ratio between tissue or cells and Lysosome Isolation Buffer ranges between 1:5 and 1:10 w/v (i.e. for 1:10, add 10 µof Lysosome Isolation Buffer per mg. of tissue). Stroke the sample 8-12 times on ice. Transfer the homogenate to a fresh tube. Add 500 µof Lysosome Enrichment Buffer. Invert the tube several times to mix. Centrifuge at 500 x g for 10 min. at 4centigrade. Collect the supernatant in a separate tube and keep on ice. Note: The number of strokes for homogenization will vary depending on the tissue type. To check lysis efficiency, place 5 µof lysate onto a glass slide, add coverslip and view

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with a microscope. Compare results with 5 µof the non-lysed cells, visualized as intact cells under the microscope. Alternatively, we recommend Trypan Blue Solution to determine the percentage of viable cells.

3. Lysosome Gradient/Lysosome Enrichment Gradient Solutions: Prepare five gradient solutions using Lysosome Gradient and Lysosome Enrichment Solution in five centrifuge tubes. Mix enough gradients for the number of samples to be assayed. For a 2 ml gradient extraction, we recommend the preparation of 375 µof every gradient containing:

Note:

We provide reagents for 50 assays using 2 ml gradient tubes or 10 assays for 20 ml gradient tubes. Required volumes will depend on the used centrifuge tube size.

4. Preparation of Discontinous Density Gradient: In an ultracentrifuge tube, prepare a discontinuous density gradient by carefully overlaying the prepared Lysosome Gradient/Lysosome Enrichment Gradient Solutions (#1 - #5). Gradients # 1 and #5 represent the top and bottom layers of the gradient respectively (See Figure a). Start preparing the discontinuous gradient by adding gradient #5. We recommend using open- or closed-top ultracentrifuge tube for this step. Do not shake or move the tubes during this process.

5. Lysosome Purification: Dilute the prepared cell or tissue lysate from Step 2, 1:4 with Lysosome Gradient, by mixing Part 1 of Lysosome Gradient with 3 Parts of lysate. Carefully add the diluted cell or tissue lysate to the top of the prepared density gradient. Centrifuge the tubes using ultracentrifuge for 2 hrs at 145,000 x g at 4centigrade. Lysosome band is visible on the top 1/10th ml of the gradient volume (see figure a). Withdraw the Lysosome Fraction band carefully by using and extra-long pipette tip (~0.2 ml) starting from top of the gradient. This fraction contains Enriched Lysosomes. To further purify, mix this fraction with 2 volume of PBS. Vortex gently. Centrifuge for 30 min. at 18,000 x g at 4centigrade. Discard the supernatant and keep the pellet containing the Purified Lysosomes.

6. Storage Conditions based on Application: For activity assay, resuspend the pellet in PBS and determine protein concentration using Bradford Method. For long term

	storage, resuspend the pellet in PBS, aliquot and snap freeze in liquid nitrogen. Transfer frozen lysosomes to -80centigrade. For the gel loading purpose, lysosomes can be stored in appropriate sample PAGE buffer (Not provided).
Sample Type	Mammalian tissues: liver, kidney, etc. Cultured Cells: suspension and adherent cells

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