Lysosome Isolation Kit from Tissue and Cultured Cells

(Catalog #BN00503; 50 Extractions; Store at -20°C)

I. Introduction:

Lysosomes are membrane-bound cell organelles found in most animal cells (except red blood cells). Lysosomes are spherical vesicles containing hydrolytic enzymes capable of breaking down different kinds of biomolecules, including proteins, nucleic acids, carbohydrates, lipids, and cellular debris. They are known to contain over 50 different enzymes, which are active under acidic conditions. Thus, lysosomes act as the waste disposal system of the cells by digesting unwanted materials in the cytoplasm. Assay Genie's 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.

II. Applications:

- Isolation of high purity lysosomes from tissues and cultured cells
- Lysosome studies like enzyme activity or uptake studies
- Western blot and ELISA

III. Sample Type:

- Mammalian tissues: liver, kidney, etc.
- Cultured Cells: suspension and adherent cells

IV. Kit Contents:

Components	BN00503	Cap Code	Part Number
Lysosome Isolation Buffer	25 ml	WM	BN00503-1
Lysosome Enrichment Buffer	100 ml	WM	BN00503-2
Lysosome Gradient	85 ml	NM	BN00503-3
Protease Inhibitor Cocktail	1 ml	Orange	BN00503-4

V. User Supplied Reagents and Equipment:

- Bench-top centrifuge with variable speed and controlled low-temperature capabilities
- Ultracentrifuge, rotor, and compatible tubes
- Glass Dounce Homogenizer
- PBS

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Thaw before use. Read the entire protocol before performing the assay.

- **Lysosome Isolation Buffer and Lysosome Enrichment Buffer**: Determine the needed volume and add Protease Inhibitor Cocktail at a ratio of 1:1000 (1 µl to 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.

VII. Lysosome Isolation 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 µl of Lysosome Isolation Buffer.

2. Procedure:

a. **Cultured Cells:** Add 500 µl of Lysosome Isolation Buffer to the pellet and vortex for 5 seconds, followed by incubation on ice for 2 min. 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 µl of Lysosome Enrichment Buffer. Invert the tube several times to mix. Centrifuge at 500 x g for 10 min. at 4°C. 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 µl of Lysosome Isolation Buffer per mg. of tissue). Stroke the sample 8-12 times on ice. Transfer the homogenate to a fresh tube. Add 500 µl of Lysosome Enrichment Buffer. Invert the tube several times to mix. Centrifuge at 500 x g for 10 min. at 4°C. 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 µl of lysate onto a glass slide, add coverslip and view with a microscope. Compare results with 5 µl of the non-lysed cells, visualized as intact cells under the microscope. Alternatively, we recommend Trypan Blue Solution (Cat. #BN00072) 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 µl of every gradient containing:

Gradient	Lysosome Gradient (µl)	Lysosome Enrichment Buffer (µl)	Final Volume (µl)	Final Gradient (%)
1	106	269	375	17
2	125	250	375	20
3	144	231	375	23
4	169	206	375	27
5	188	187	375	30

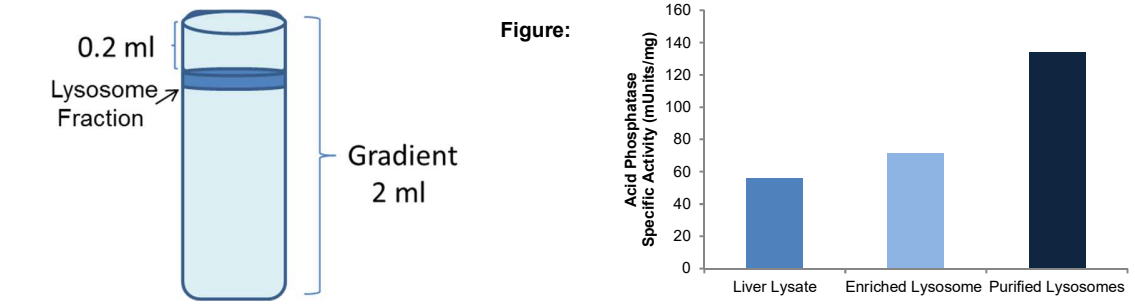
Note:

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 4°C. 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 4°C. 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 -80°C. For the gel loading purpose, lysosomes can be stored in appropriate sample PAGE buffer (Not provided).

(a) (b)

Figure:



Lysosome Isolation: (**a.**) Tentative Lysosome band location in gradient density. (**b.**) Acid Phosphatase (biomarker enzyme of lysosomes) specific activity increased with each purification step. Activity Assay experiments were carried out using Acid Phosphatase Activity Colorimetric Assay Kit. Data is normalized by the amount of protein used per well.

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