

Technical Bulletin

Lysosome Isolation Kit

Catalog number MAK405

Product Description

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.

The Lysosome Isolation Kit provides a procedure for isolating an enriched or purified lysosomal fraction from animal tissues and cultured cells by differential centrifugation followed by density gradient centrifugation.

The kit is suitable for the isolation of high purity lysosomes from mammalian tissues (e.g., liver, kidney, etc.) and cultured cells (suspension or adherent).

Components

The kit is sufficient for 50 extractions

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|---|--------|
| • Lysosome Isolation Buffer
Catalog Number MAK405A | 25 mL |
| • Lysosome Enrichment Buffer
Catalog Number MAK405B | 100 mL |
| • Lysosome Gradient
Catalog Number MAK405C | 85 mL |
| • Protease Inhibitor Cocktail
Catalog Number MAK405D | 1 mL |

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of $RCF \geq 600 \times g$
- Refrigerated ultracentrifuge capable of $RCF \geq 145,000 \times g$, rotor, and compatible tubes
- Phosphate buffered saline (PBS) (Catalog Number P3813 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at $-20\text{ }^{\circ}\text{C}$, protected from light.

Preparation Instructions

Thaw components before use.

Lysosome Isolation Buffer and Lysosome Enrichment Buffer: Determine the needed volumes. Add Protease Inhibitor Cocktail at a ratio of 1:1000 (1 μL to 1 mL Buffer) to Lysosome Isolation Buffer and Lysosome Enrichment Buffer.

Procedure

All samples and standards should be run in duplicate.

Note: Prior to beginning procedure, place centrifuge rotor, centrifuge tubes, reagents, and homogenizer on ice or in refrigerator.

Sample Preparation

Cultured Cells

1. Pellet 2×10^7 cells by centrifugation at $600 \times g$ for 10 minutes.
2. Carefully remove and discard the supernatant.

Tissues

1. Isolate the tissue of interest (~100 mg).
2. Immerse the tissue sample in 1 mL of ice-cold PBS.
3. Rinse the tissue twice in 1 mL of PBS to remove blood.
4. Mince the tissue on ice into small pieces using scissors.
5. Discard the PBS used for mincing.
6. Add 800 μ L of Lysosome Isolation Buffer.

Homogenization

Cultured Cells

1. Add 500 μ L of Lysosome Isolation Buffer to the pellet and vortex for 5 seconds, followed by incubation on ice for 2 minutes.
2. Homogenize the cells using a precooled glass Dounce Homogenizer.
3. Stroke the sample 20-30 times on ice.
4. Transfer the homogenate to a fresh tube.
5. Add 500 μ L of Lysosome Enrichment Buffer.
6. Invert the tube several times to mix.
7. Centrifuge at $500 \times g$ for 10 minutes at 4 °C.
8. Collect the supernatant in a separate tube and keep on ice.

Tissue

1. Homogenize the tissue using a precooled glass Dounce homogenizer.
2. Add Lysosome Isolation Buffer. The optimal ratio between tissue and Lysosome Isolation Buffer is between 1:5 and 1:10 (w/v) (i.e. for 1:10, add 10 μ L of Lysosome Isolation Buffer per mg of tissue).
3. Stroke the sample 8-12 times on ice. The number of strokes for homogenization will vary depending on the tissue type.

To check lysis efficiency, place 5 μ L of lysate onto a glass slide, add coverslip and view with a microscope. Compare results with 5 μ L of the non-lysed cells, visualized as intact cells under the microscope.

Alternatively, use Trypan Blue Solution to determine the percentage of viable cells.

4. Transfer the homogenate to a fresh tube.
5. Add 500 μ L of Lysosome Enrichment Buffer.
6. Invert the tube several times to mix.
7. Centrifuge at $500 \times g$ for 10 minutes at 4 °C.
8. Collect the supernatant in a separate tube and keep on ice.

Lysosome Gradient/Lysosome Enrichment Gradient Solutions

Prepare five gradient solutions (GS #1 - #5) 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, prepare 375 μ L of every gradient solution according to Table 1.

Note: The kit provides enough reagents for 50 extractions using 2 mL gradient tubes or 10 assays for 20 mL gradient tubes. Required volumes will depend on the used centrifuge tube size.



Table 1.

Preparation of Lysosome Gradient/Lysosome Enrichment Gradient Solutions

GS#	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

Preparation of Discontinuous Density Gradient

In an ultracentrifuge tube, prepare a discontinuous density gradient by carefully overlaying the prepared Lysosome Gradient/Lysosome Enrichment Gradient Solutions (GS #1 - #5). Gradients GS #1 and #5 represent the top and bottom layers of the gradient respectively (see Figure 1).

Start preparing the discontinuous gradient by adding Gradient GS #5. Open- or closed-top ultracentrifuge tubes are recommended for this step.

Do not shake or move the tubes during this process.

Lysosome Purification

1. Dilute the prepared cell or tissue lysate 1:4 with Lysosome Gradient, by mixing 1 part of Lysosome Gradient with 3 parts of lysate.
2. Carefully add the diluted cell or tissue lysate to the top of the prepared density gradient.
3. Centrifuge the tubes using ultracentrifuge for 2 hours at $145,000 \times g$ at 4 °C.
4. The lysosome band is visible on the top 1/10th mL of the gradient volume (see Figure 1).

5. Withdraw the Lysosome Fraction band carefully by using an extra-long pipette tip (~0.2 mL) starting from top of the gradient. This fraction contains Enriched Lysosomes.
6. To further purify, mix this fraction with 2 volumes of PBS (not included). Vortex gently.
7. Centrifuge for 30 minutes at $18,000 \times g$ at 4 °C.
8. Discard the supernatant and keep the pellet containing the Purified Lysosomes.

Storage Conditions based on Application

For activity assay, resuspend the pellet in PBS (not included) and determine protein concentration using Bradford Method (not included)

For long term storage, resuspend the pellet in PBS, aliquot and snap freeze in liquid nitrogen. Transfer frozen lysosomes to -80 °C.

For gel loading purposes, lysosomes can be stored in appropriate sample PAGE buffer (not provided).

Figure 1.
Typical lysosome band location in
gradient density

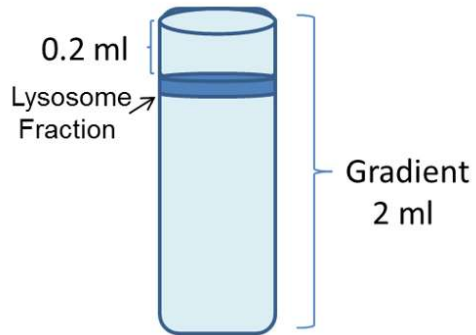
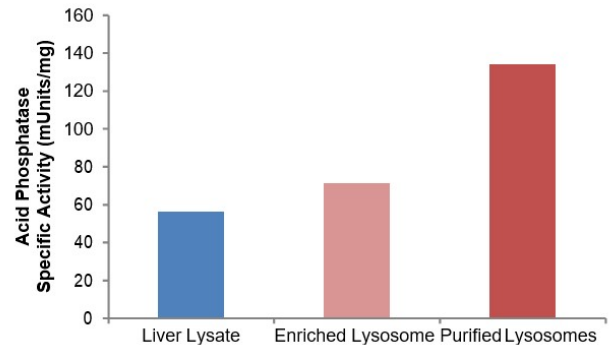


Figure 2.
Acid Phosphatase (biomarker enzyme of
lysosomes) specific activity increased with
each purification step. Data is normalized by
the amount of protein used per well.
Lysosomes were isolated and purified
following the kit protocol.



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