Version 2 Last updated 13 October 2023

ab234047
Lysosome Isolation Kit

For the isolation of high purity lysosomes from animal tissues and cultured cells.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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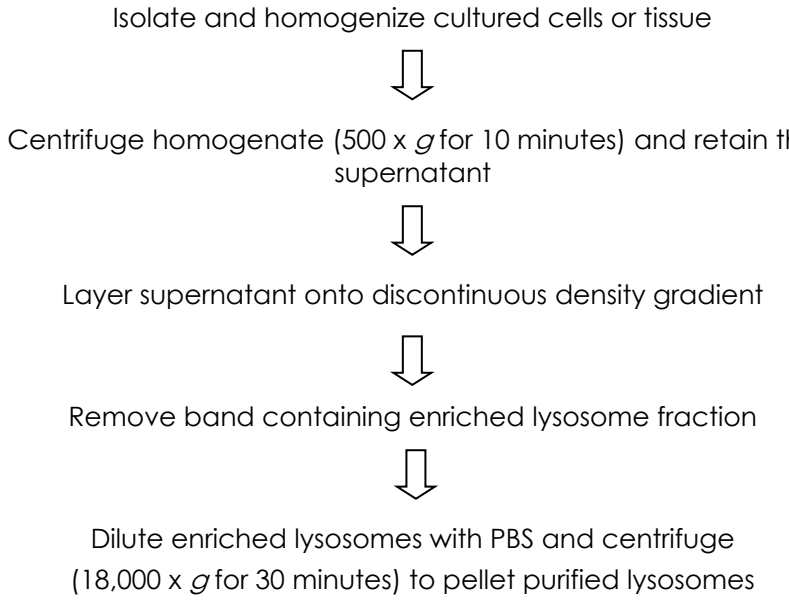
1. Overview

Lysosome Isolation Kit (ab234047) 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.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Lysosome Isolation Buffer	25 mL	-20°C	-20°C
Lysosome Enrichment Buffer	100 mL	-20°C	-20°C
Density Gradient Media II/Lysosome Gradient	85 mL	-20°C	-20°C
Protease Inhibitor Cocktail II/Protease Inhibitor Cocktail	1 mL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Lysosome Isolation Buffer

1. Thaw before use.
2. Determine the volume of Lysosome Isolation Buffer needed and add Protease Inhibitor Cocktail II/Protease Inhibitor Cocktail at a ratio of 1:1000 (1 µL to 1 mL Lysosome Isolation Buffer).

5.2 Lysosome Enrichment Buffer

1. Thaw before use.
2. Determine the volume of Lysosome Enrichment Buffer needed and add Protease Inhibitor Cocktail II/Protease Inhibitor Cocktail at a ratio of 1:1000 (1 µL to 1 mL Lysosome Enrichment Buffer).

5.3 Density Gradient Media II/Lysosome Gradient

Ready to use as supplied.

5.4 Protease Inhibitor Cocktail II/Protease Inhibitor Cocktail

Ready to use as supplied.

Δ Note: *See the Isolation procedure (Section 7) for details of how to prepare the discontinuous density gradient.*

6. Sample Preparation

General sample information:

Use fresh cells or tissue.

Precool ultracentrifuge and accessories: Place rotor, tubes, reagents, and dounce homogenizer on ice/refrigerator.

6.1 Cultured Cells:

1. Pellet 2 x10^7 cells by centrifugation at 600 x *g* for 10 minutes.
2. Carefully remove and discard the supernatant.

6.2 Tissue:

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

7. Isolation Procedure

– Equilibrate all materials and prepared reagents to 4°C prior to use and gently agitate.

7.1 Homogenization

Cultured cells

1. Add 500 µL 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. Stroke the sample 20-30 times on ice.
3. Transfer the homogenate to a fresh tube. Add 500 µL of Lysosome Enrichment Buffer.
4. Invert the tube several times to mix.
5. Centrifuge at 500 x *g* for 10 minutes at 4°C. Collect the supernatant in a separate tube and keep on ice.

Tissue

1. Homogenize the tissue using a precooled glass Dounce 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).
2. Stroke the sample 8-12 times on ice.
3. Transfer the homogenate to a fresh tube. Add 500 µL of Lysosome Enrichment Buffer.
4. Invert the tube several times to mix.
5. Centrifuge at 500 x *g* for 10 minutes at 4°C. Collect the supernatant in a separate tube and keep on ice.

Δ 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 to determine the percentage of viable cells.*

7.2 Density Gradient Media II/Lysosome Gradient & Lysosome Enrichment Buffer Gradient Solutions:

- Prepare five gradient solutions using Density Gradient Media II/Lysosome Gradient and Lysosome Enrichment Buffer 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	Density Gradient Media II/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

Δ 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 size of centrifuge tube used.*

7.3 Discontinuous Density Gradient:

1. In an ultracentrifuge tube, prepare a discontinuous density gradient by carefully overlaying the prepared Density Gradient Media II/Lysosome Gradient & Lysosome Enrichment Gradient Solutions.
2. Gradients 1 and 5 represent the top and bottom layers of the gradient, respectively.
3. Start preparing the discontinuous gradient by adding gradient 5. We recommend using either open- or closed-top ultracentrifuge tubes for this step. Do not shake or move the tubes during this process.

7.4 Lysosome purification:

1. Mix the prepared cell or tissue lysate (see Section 6) with Density Gradient Media II/Lysosome Gradient, by mixing 4 parts of cell/tissue lysate with 1 part of Density Gradient Media II/Lysosome Gradient.
2. Carefully add the diluted cell or tissue lysate to the top of the prepared density gradient.
3. Centrifuge the tubes using an ultracentrifuge for 2 hours at 145,000 x *g* at 4°C.
4. The lysosome band is visible in the top 1/10th of the gradient volume.
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. Vortex gently. Centrifuge for 30 minutes at 18,000 x *g* at 4°C. Discard the supernatant and keep the pellet containing the purified lysosomes.

7.5 Storage of purified lysosomes:

1. For activity assays, resuspend the pellet in PBS and determine the protein concentration using the Bradford method.
2. For long term storage, resuspend the pellet in PBS, aliquot and snap freeze in liquid nitrogen. Store frozen lysosomes at -80°C.
3. For the samples to be analyzed by SDS-PAGE/Western blot, lysosomes can be stored in appropriate sample SDS-PAGE buffer.

8. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only**.

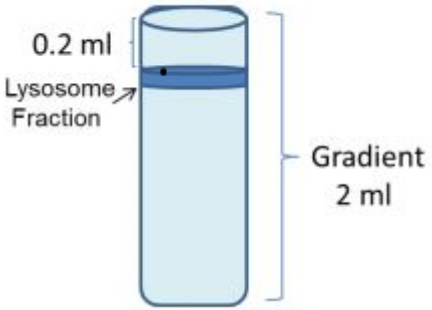


Figure 1. Location of lysosome band in Discontinuous Density Gradient following ultracentrifugation.

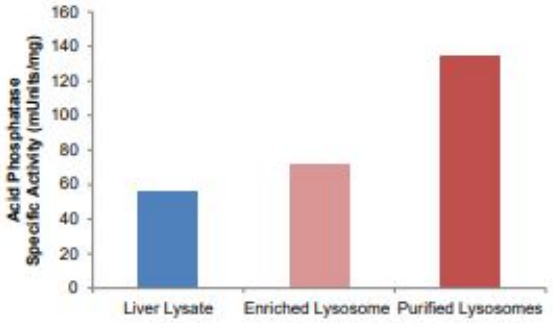


Figure 2. Acid Phosphatase (biomarker enzyme for lysosomes) specific activity (mU/mg protein) increases with each purification step. Data is normalized by the amount of protein used per well.

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)