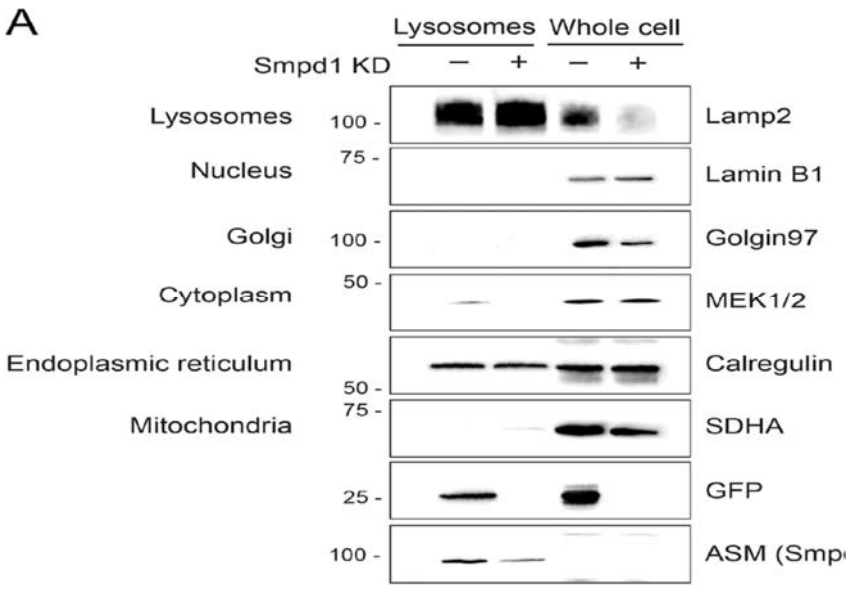
Lysosome Isolation by A Spin Column-Based Technology

By Invent Biotechnologies Inc.

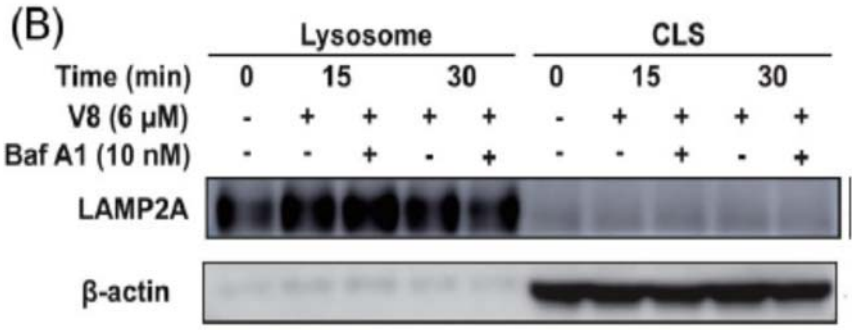
Lysosomes are organelles enclosed by membranes that play a role in breaking down and recycling cellular materials. However, isolating lysosomes is challenging due to their fragility, small size, and scarcity, as well as the risk of contamination from other organelles. Various methods have been developed to isolate lysosomes, each with its limitations. Density gradient centrifugation is a widely used method where lysosomes are separated based on density. Unfortunately, this approach can lead to contamination with similar organelles in buoyant density, such as mitochondria or peroxisomes. Moreover, lysosomes are highly sensitive to pH and osmolarity changes, which can cause them to rupture during isolation. Other methods include immunoprecipitation using lysosomal markers like LAMP-1 or LAMP-2, as well as subcellular fractionation through differential centrifugation. However, these techniques may also result in contamination, or non-specific binding of the lysosomal markers. Another approach involves using lysosome-specific dyes like LysoTracker or acridine orange, followed by fluorescence-activated cell sorting (FACS). However, this method necessitates live cells and can compromise lysosomal function due to the dye-loading process (1).

To overcome the limitations of existing methods, we have developed a simple and rapid kit (Cat# LY-034) for isolating lysosomes from cultured cells and tissues. This kit utilizes a spin column-based cell rupturing technology instead of a Dounce homogenizer. By combining the spin column with differential centrifugation and preferential precipitation, we can achieve high purity in less than one hour. The isolated lysosomes remain intact and in their native form, making them suitable for various downstream analyses.

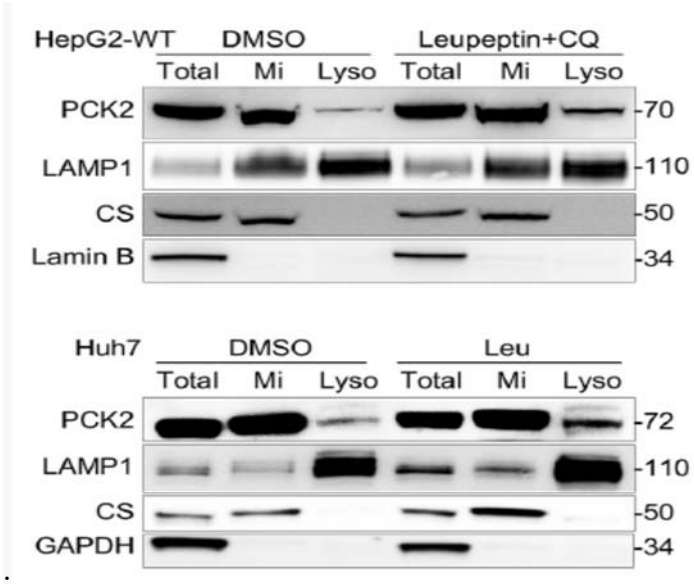
In a study conducted by Ravodina A. M. et al. (2), lysosomes were isolated from Smpd1 knock out RAW cells using the LY-034 kit. The researchers then examined the presence of major organelles using specific markers. As shown in the Figure below, the lysosome marker LAMP2 is significantly enriched compared to the whole cell lysate. The isolated lysosomes are free from contamination by nuclear, Golgi apparatus, mitochondrial, and cytosolic proteins.



In an independent study (3), researchers isolated a lysosomal fraction from Jurkat cells and conducted Western blotting analysis. As evidenced by the diagram below, the lysosomal marker LAMP2A exhibited a significant enrichment compared to the total cell lysate (CLS). The presence of cytosolic proteins was minimal, indicating minimal cross-contamination.

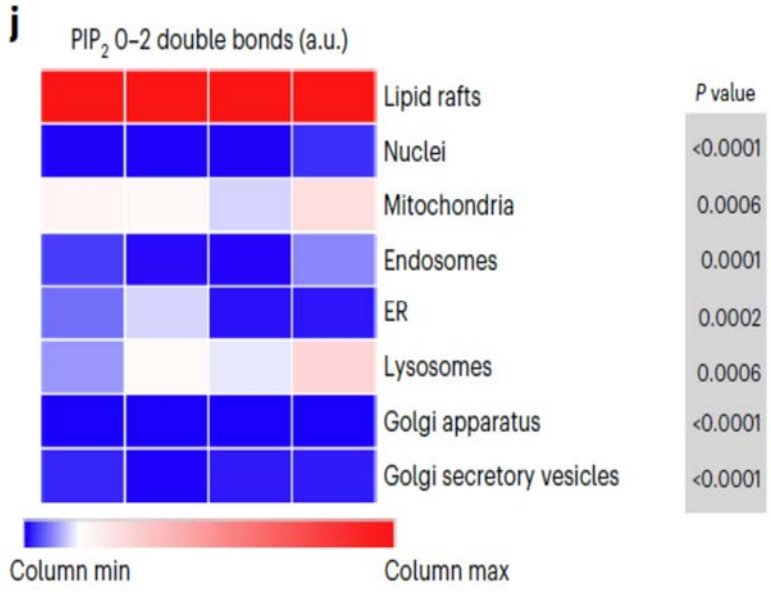


The impressive efficacy of LY-034 is further exemplified in a study conducted by Guo, Q.Y. et al. (4). The researchers investigated the subcellular localization of PCK2 in HepG2 and Huh7 cells using Western blotting. Lysosomes were isolated using LY-034 and compared to the total cell lysate. The results indicate a significant enrichment of the lysosomal marker in comparison to the cell lysate, with no cross-contamination observed from nuclear and cytosolic fractions.
The study revealed that PCK2 primarily localizes in the mitochondria.



In addition to Western blotting, lysosomes isolated using LY-034 have also been utilized for mass spectrometry analysis. Gordevicius, J. et al. (5) conducted a study investigating the relationship between epigenetic inactivation of the autophagy-lysosomal system in the appendix and Parkinson's disease. Isolated lysosomes from the appendix were subjected to tandem mass tag (TMT) quantitative proteomic analysis, successfully validating the epigenetic findings.

While isolated lysosomes are commonly used for protein analysis, recent research has demonstrated the suitability of lysosomes isolated using LY-034 for lipidomic analysis (6). In this particular study, lysosomes were isolated from CD8+ cells, and the lipids were extracted using a biphasic tert-butyl ether extraction protocol. The extracted lipids underwent lipidomic analysis using LC-MS/MS. The Figure below presents a heat map comparing polyunsaturated phosphatidylinositol biphosphate (PIP2) in lysosomes isolated using LY-034 with other organelles isolated using different kits from our company (lipid raft, mitochondria, endosomes, Golgi apparatus, endoplasmic reticulum, and nuclei). The results demonstrate a clean and distinct differentiation.



In conclusion, the isolation of lysosomes continues to pose challenges. However, our spin-column-based lysosome isolation kit offers a novel technology that is simple, rapid, and delivers consistent and reproducible results. Lysosomes isolated using LY-034 maintain their integrity and native form, making them suitable for protein and lipid analysis via Western blotting and mass spectrometry. Furthermore, ongoing investigations are exploring additional applications for this innovative method.

References:

1. Saftig, P., & Klumperman, J. (2009). Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nature Reviews Molecular Cell Biology, 10(9), 623-635.
2. Ravodina, A. M., Badgeley, M. A., Rajagopalan, S., Fedyukina, D. V., & Maiseyeu, A. (2020). Facile Cholesterol Loading with a New Probe ezFlux Allows for Streamlined Cholesterol Efflux Assays. *ACS Omega*.

3. Qing, Y., Guo, Y., Zhao, Q., Hu, P., Li, H., Yu, X., ... & Hui, H. (2023). Targeting lysosomal HSP70 induces acid sphingomyelinase-mediated disturbance of lipid metabolism and leads to cell death in T cell malignancies. *Clinical and Translational Medicine*, *13*(3), e1229.
4. Jing, Z., Gao, J., Li, J., Niu, F., Tian, L., Nan, P., ... & Zhao, X. (2021). Acetylation-induced PCK isoenzyme transition promotes metabolic adaption of liver cancer to systemic therapy. *Cancer Letters*.
5. Gordevicius, J., Li, P., Marshall, L. L., Killinger, B. A., Lang, S., Ensink, E., ... & Labrie, V. (2021). Epigenetic inactivation of the autophagy–lysosomal system in appendix in Parkinson's disease. *Nature Communications*, *12*(1), 1-18.
6. Edwards-Hicks, J., Apostolova, P., Buescher, J. M., Maib, H., Stanczak, M. A., Corrado, M., ... & Pearce, E. L. (2023). Phosphoinositide acyl chain saturation drives CD8+ effector T cell signaling and function. *Nature Immunology*, 1-15.