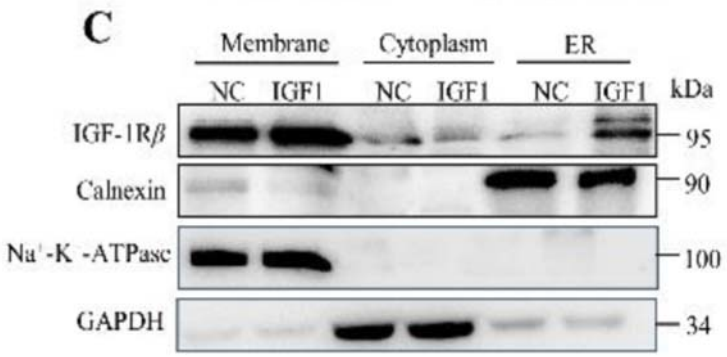
ER Isolation Using a Spin Column-Based Technology

(Invent Biotechnologies Inc.)

The endoplasmic reticulum (ER) is a highly intricate organelle composed of membranes that plays a critical role in protein synthesis, folding, and lipid metabolism. To comprehend its structure and function, it is essential to isolate the ER from cells. Density gradient centrifugation is a commonly employed method for ER isolation, which separates the ER from other components within the cell based on its buoyant density (1). However, this technique presents challenges due to the potential contamination of the ER by other organelles and debris, leading to interference in subsequent analysis. One of the main obstacles faced when using density gradient centrifugation for ER isolation is the heterogeneity of ER subdomains, namely the rough ER and smooth ER, which possess distinct densities and compositions. Consequently, this can result in overlapping gradients and difficulties in accurately separating and identifying specific ER populations (2). Moreover, the ER is sensitive to the harsh conditions associated with density gradients, such as high salt concentrations or detergents. These conditions can cause the loss of protein or lipid components, ultimately altering the properties of the ER. Additionally, the choice of labeling and purification methods can impact the effectiveness and specificity of ER isolation. For example, certain labeling techniques may lead to non-specific labeling of other cellular components or require harsh treatments that modify the properties of the ER (3).

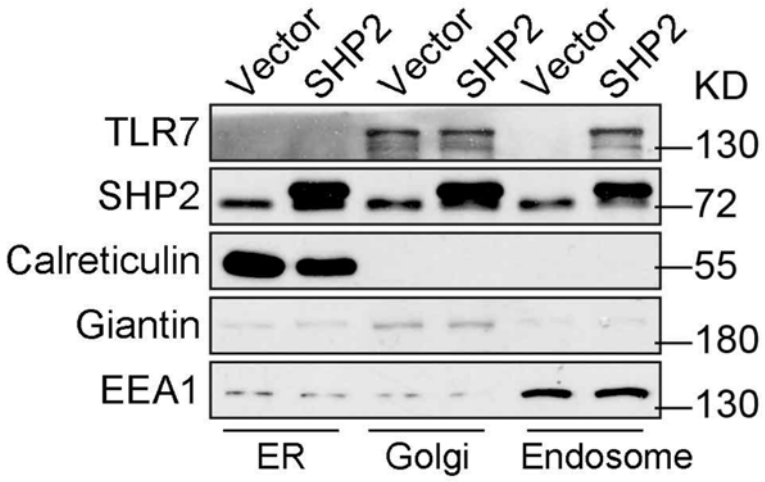
We have developed a spin-column-based ER isolation kit (Cat# ER-036) to address these challenges. This kit offers a simple and straightforward method for isolating highly enriched ER. It eliminates the need for a Dounce homogenizer and ultracentrifugation by utilizing a combination of spin columns, differential centrifugation, and preferential precipitation. The entire protocol can be completed in approximately 90 minutes. The efficacy of this kit has been validated through numerous high impact factor publications.

One such study by Li et al. (4) investigated the mechanism of autophagy in HepG2 cells using the ER-036 kit. In this research, ER was isolated from the cell line and subjected to analysis via Western blotting. The study aimed to compare IGF-1R signals in the ER fraction with those obtained from isolated cell membranes and cytoplasm. The results, depicted in the below Figure, exhibited a significant enhancement of ER signals in the ER fraction (identified by Calnexin), while demonstrating minimal cross-contamination from the plasma membrane (Na-K-ATPase) and cytoplasm fraction (GAPDH).

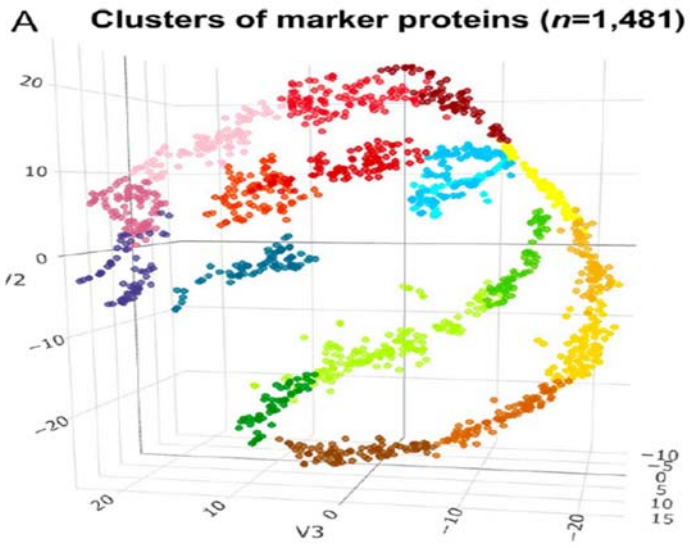


The exceptional efficacy of ER-036 is reaffirmed in the Figure below (5). The study involved the isolation of ER from THP1 cells, followed by probing with antibodies specific to TLR7, SHP2, ER, Golgi, and

endosomes using Western blotting. As anticipated, strong calreticulin signals (a marker of the ER) were exclusively detected in the ER fraction, with minimal cross-contamination from Golgi and endosomes. Notably, the Golgi apparatus and endosomes were isolated using separate kits offered by our company (Cat# SM-005 and ED-028, respectively).

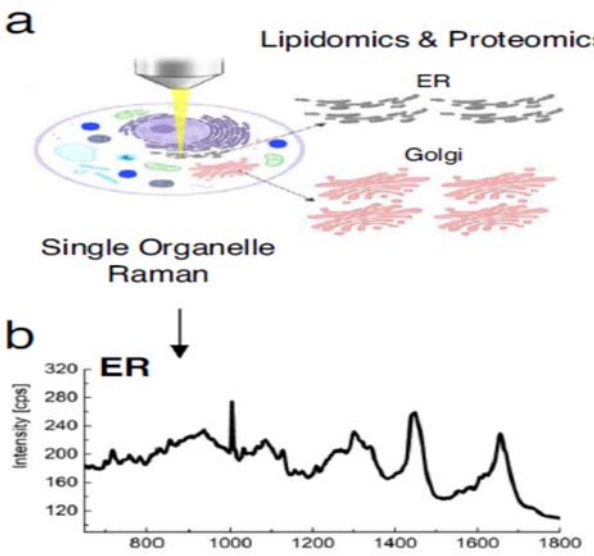


Apart from Western blotting, the application of ER-036 in ER isolation has extended to mass spectrometry analysis. Huang et al. (6) conducted a study focused on characterizing the subcellular protein localization in a liver cancer cell line. They employed mass spectrometry to compare membrane-bound organelles and successfully identified 1481 marker proteins. Furthermore, they generated three-dimensional visualizations for 18 clusters of these marker proteins. For the isolation of endosomes (ED-028), Golgi apparatus (GO-037), lysosomes (LY-034), and plasma membrane (SM-005) in this study, kits supplied by Invent Biotechnologies, Inc. were utilized.

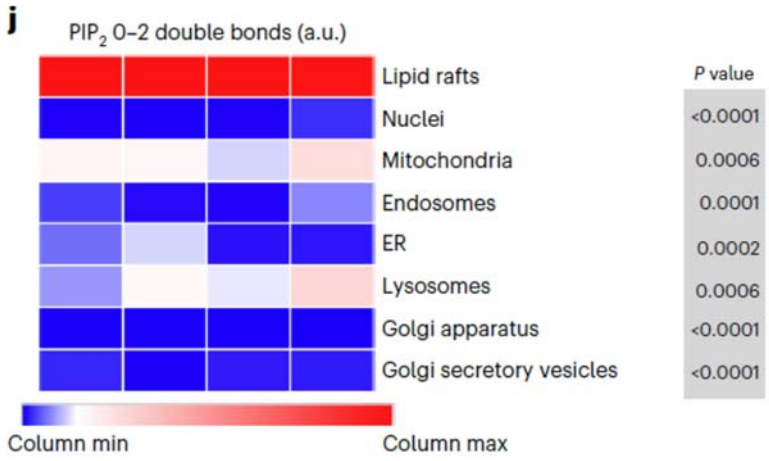


In addition to protein analysis through Western blotting and mass spectrometry, the utilization of ER-036 for ER isolation has also proved successful in lipidomic analysis. Two studies conducted by Lita et al. (7) and Dowdy et al. (8) explored the role of sphingolipids in the pathogenesis of glioma using isolated ER.

These studies discovered that elevated levels of specific lipids within the sphingolipid pathway can serve as potential targets for the development of metabolic therapies. In these investigations, ER-036 was employed to isolate ER from tumor cells. Subsequently, lipids were extracted from the isolated ER and subjected to lipidomic analysis using Mass Spectrometry.



In a separate investigation (9), ER isolation was performed on CD8+ cells, and a biphasic tert-butyl ether extraction protocol was employed to extract lipids. The extracted lipids were then subjected to lipidomic analysis using LC-MS/MS. The accompanying figure displays a heat map comparing polyunsaturated phosphatidylinositol biphosphate (PIP2) with various other organelles, which were isolated using different kits provided by our company (lipid raft LR-042, mitochondria MP-007, endosomes ED-028, Golgi apparatus GO-037, lysosomes LY-034, and nuclei). The results obtained from this analysis were found to be precise and distinct, offering a clear representation of the lipid distribution.



In summary, the isolation of ER continues to present challenges, but our spin-column-based ER isolation kit offers a novel and efficient solution. This kit utilizes a straightforward and time-efficient approach, delivering consistent and reproducible results. The ER isolated using ER-036 maintains its integrity and

native form, making it suitable for protein and lipid analysis through techniques such as Western blotting and mass spectrometry. Furthermore, ongoing research is exploring additional applications for this kit, expanding its potential applications in the field.

References:

1. Voeltz, G. K., & Prinz, W. A. (2007). Sheets, ribbons and tubules—how organelles get their shape. Nature Reviews Molecular Cell Biology, 8(3), 258-264.
2. Shemesh, T., Klemm, R. W., Romano, F. B., Wang, S., Vaughan, J., Zhuang, X., & Tukachinsky, H. (2014). A model for the generation and interconversion of ER morphologies. Proceedings of the National Academy of Sciences, 111(49), E5243-E5251.
3. Carrasco, S., & Meyer, T. (2011). STIM proteins and the endoplasmic reticulum-plasma membrane junctions. Annual review of biochemistry, 80, 973-1000.
4. Li, Y., Li, K., Pan, T., Xie, Q., Cheng, Y., Wu, X., ... & Cui, S. (2023). Translocation of IGF-1R in endoplasmic reticulum enhances SERCA2 activity to trigger Ca2+ ER perturbation in hepatocellular carcinoma. *Acta Pharmaceutica Sinica B*.
5. Zhu, Y., Shao, F., Yan, W., Xu, Q., & Sun, Y. (2020). Inhibition of SHP2 ameliorates psoriasis by decreasing TLR7 endosome localization. *medRxiv*.
6. Huang, F., Tang, X., Ye, B., Wu, S., & Ding, K. (2022). PSL-LCCL: a resource for subcellular protein localization in liver cancer cell line SK_HEP1. *Database*, *2022*.
7. Lita, A., Pliss, A., Kuzmin, A., Yamasaki, T., Zhang, L., Dowdy, T., ... & Larion, M. (2021). IDH1 mutations induce organelle defects via dysregulated phospholipids. *Nature Communications*, *12*(1), 1-16.
8. Dowdy, T., Zhang, L., Celiku, O., Movva, S., Lita, A., Ruiz-Rodado, V., ... & Larion, M. (2020). Sphingolipid Pathway as a Source of Vulnerability in IDH1mut Glioma. *Cancers*, *12*(10), 2910.
9. 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.