A Novel Technology for Isolation of Golgi Apparatus

(Invent Biotechnologies Inc.)

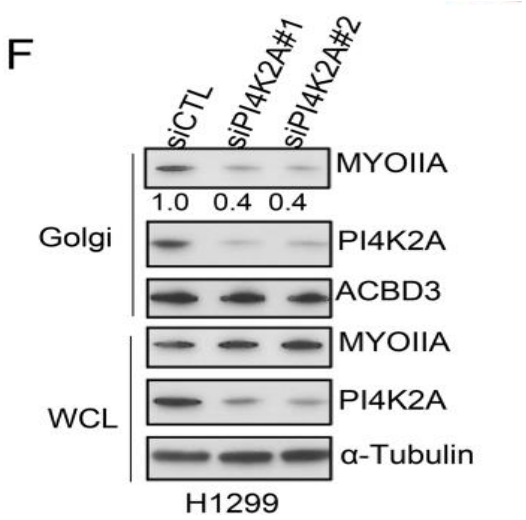
The Golgi apparatus is a complex organelle found in eukaryotic cells, responsible for sorting, modifying, and transporting proteins. To understand its structure and function, Golgi isolation is necessary. Density gradient centrifugation is a commonly used method that separates the Golgi based on its density. However, this approach presents challenges, including contamination from other organelles and debris, which can interfere with further analysis.

A major difficulty with density gradient centrifugation is the heterogeneity of the Golgi sub-compartments, such as the cis-Golgi, medial-Golgi, and trans-Golgi. These sub-compartments differ in density and composition, leading to overlapping gradients and complications in accurately separating and identifying specific Golgi populations (1). Additionally, the Golgi is sensitive to the harsh conditions of the gradient, risking the loss of Golgi enzymes or alterations in its properties. The choice of labeling and purification methods also impacts the effectiveness and specificity of Golgi isolation. Some labeling techniques may result in non-specific labeling of other cellular components or require harsh treatments that modify Golgi properties (2).

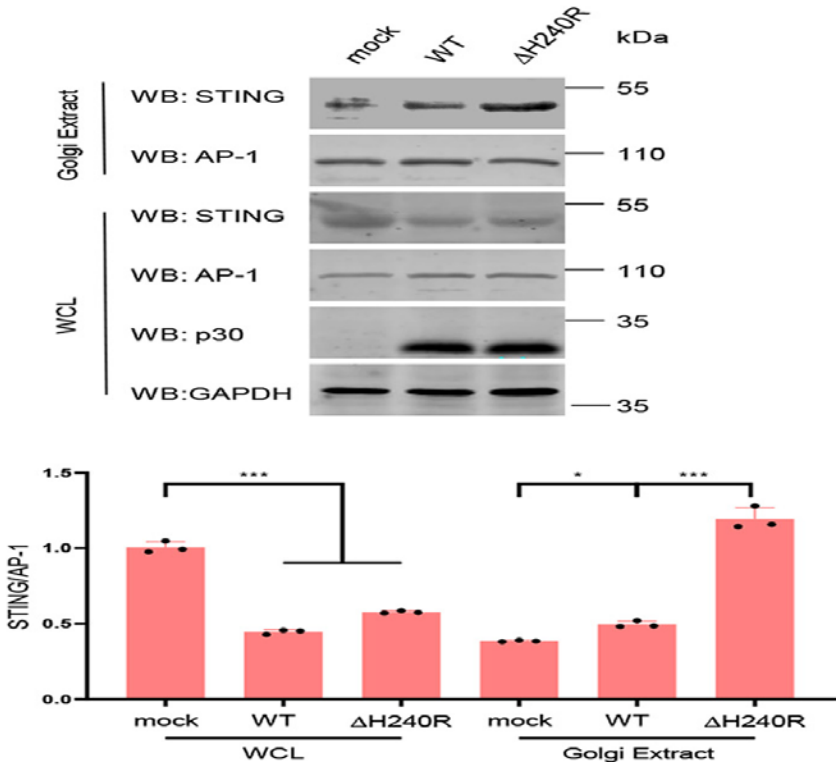
To overcome these challenges, alternative methods for Golgi isolation have been developed, including immunoaffinity purification and membrane filtration. These methods offer higher specificity and accuracy compared to density gradient centrifugation (3). However, they are often time-consuming and require specialized equipment, resulting in the need for large sample sizes and the potential for cross-contamination.

To address these limitations, we have developed a spin column-based kit (Cat# GO-037) that allows for rapid isolation of the Golgi apparatus from cultured cells and tissues. This kit enables the isolation of both cis and trans Golgi compartments. The following data demonstrates consistent and reproducible results, providing a solution to the challenges associated with Golgi isolation.

In their study, Tan et al. (4) utilized the GO-037 kit for the isolation of the Golgi apparatus from lung cancer cells to investigate the mechanism of the P14K2-dependent switch that triggers P14P synthesis in both Golgi and endosomes. The data obtained from their research reveal notable differences in the expression levels of MYOIIA between the Golgi fraction and the total cell lysate. However, they observed similar expression levels of P14K2A in siRNA-transfected H1299 cells.

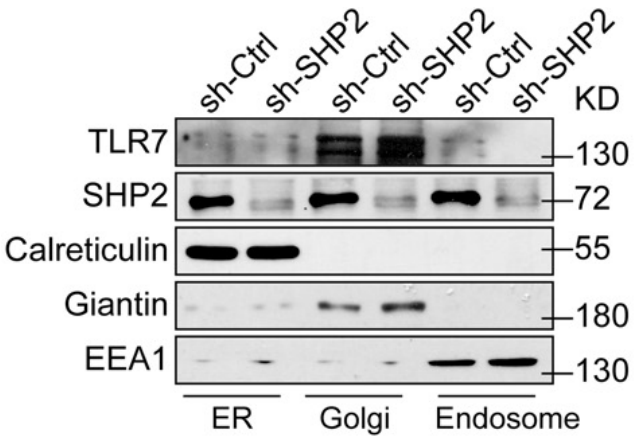


Ye et al. (5) conducted a study where they employed the GO-037 kit to extract the Golgi apparatus from primary porcine alveolar macrophages. Their objective was to semi-quantify the STING protein within both the Golgi apparatus and the whole cell lysate. The results demonstrated a significant elevation in the expression of the STING protein specifically within the Golgi apparatus when compared to the whole cell lysate. Furthermore, their findings indicate that the STING protein plays a role in interacting with the H240R protein, effectively inhibiting its oligomerization and preventing its translocation from the endoplasmic reticulum to the Golgi apparatus.



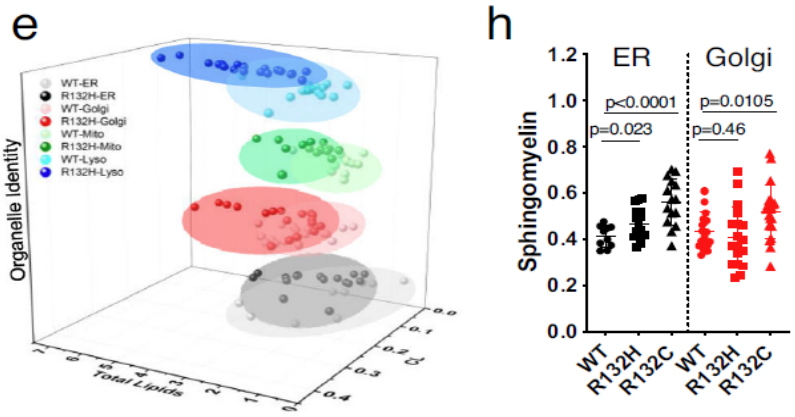
In order to address the interconnectedness of intracellular organelles, we have developed a diverse range of innovative kits for the isolation of specific organelles. These kits include the isolation of the plasma membrane (Cat# SM-005), endosomes (Cat# ED-028), lysosomes (Cat# LY-034), endoplasmic reticulum (Cat# ER-036), mitochondria (Cat# MP-007), as well as lipid rafts (Cat# LR-039 and LR-042), among others. Our kits are specifically designed to facilitate the study of protein trafficking mechanisms within cells. It is common in many research publications to employ multiple kits simultaneously, enabling the investigation of various aspects of organelle dynamics and interplay.

The exceptional performance of the GO-037 kit and other kits is demonstrated in the below Figure (5). In this particular study, Golgi apparatus, ER, and endosomes were isolated from THP1 cells and analyzed using Western blotting with TLR7, SHP2, ER, Golgi, and endosome antibodies. As expected, minimal cross-contamination was observed among the three organelle preparations. To validate the isolation process, organelle markers such as calreticulin for the ER, giantin for the Golgi, and EEA1 for the endosome were utilized. These results highlight the reliability and effectiveness of our kits in obtaining pure organelle fractions for downstream analysis.



Lita et al. (6) focused on investigating the involvement of sphingolipids in the pathogenesis of glioma. To accomplish this, they utilized the GO-037 kit for the isolation of the Golgi apparatus and the ER-036 kit for the isolation of the endoplasmic reticulum. Their findings revealed that elevated levels of specific lipids within the sphingolipid pathway could potentially serve as targets for the development of metabolic therapies.

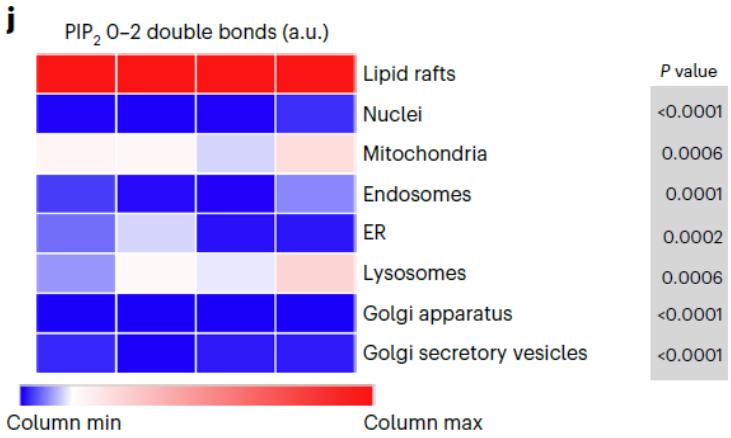
To analyze these lipids, the Golgi apparatus was isolated from tumor cells using the GO-037 kit, followed by lipid extraction. Subsequently, the extracted lipids were subjected to lipidomic analysis using mass spectrometry. This comprehensive approach allowed for a detailed examination of the lipid composition and provided valuable insights into the potential mechanisms underlying glioma pathogenesis and the development of therapeutic strategies.



In another study (7), both trans-Golgi and Golgi secretary vesicles were isolated from CD8+ cells. A biphasic tert-butyl ether extraction protocol was employed to extract the lipids from these vesicles. Subsequently, the extracted lipids underwent lipidomic analysis using LC-MS/MS.

To investigate the distribution of polyunsaturated phosphatidylinositol biphosphate (PIP2) in the Golgi apparatus, a heat map was generated. This heat map was then compared with the PIP2 levels in other organelles using specific organelle isolation kits provided by our company.

The obtained results were found to be highly conclusive and unambiguous, providing a clear understanding of the distribution of PIP2 within the Golgi apparatus compared to other organelles. The use of our specific organelle isolation kits ensured clean and reliable data for accurate analysis.



To summarize, density gradient centrifugation is a valuable technique for Golgi isolation; however, it requires careful protocol optimization and consideration of potential challenges to obtain pure and high-quality Golgi samples. In contrast, our spin-column-based technology offers a straightforward and fast approach for isolating the Golgi apparatus from cultured cells and tissues, yielding consistent and dependable results. Golgi samples obtained using our GO-037 kit are preserved in their intact and native form, making them suitable for protein and lipid analysis.

Click here for a complete list of Minute™ Cell Fractionation Kits.

References:

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