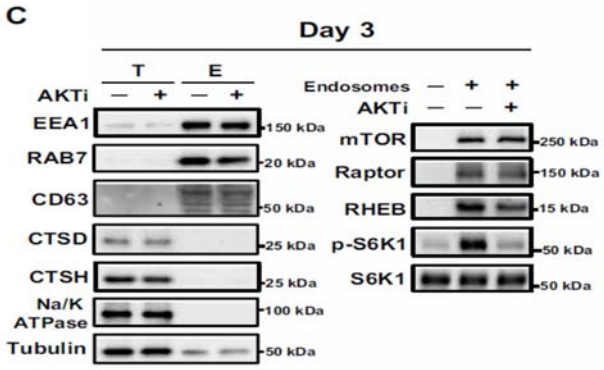
# A Novel Technology for Isolation of Endosomes

***(Invent Biotechnologies Inc.)***

The isolation of endosomes plays a vital role in studying endocytosis and intracellular trafficking. However, this process can be challenging due to the complexity of cellular systems and the heterogeneous nature of endosomes. One commonly used method for endosome isolation is density gradient centrifugation, which separates endosomes from other cellular components based on their density. Although density gradient centrifugation is a valuable tool, it has limitations that can affect its efficiency and specificity. Contamination by other organelles or debris is a significant challenge, as it can interfere with downstream analysis. To minimize contamination and maximize endosome purity, careful optimization of gradient conditions, including buffer composition and centrifugation speed, is necessary. Another challenge is the heterogeneity of endosome subpopulations, which vary in size, composition, and density. This can lead to overlapping gradients and difficulties in accurately separating and identifying specific endosome populations. Moreover, endosomes can be sensitive to the harsh conditions of the gradient, potentially resulting in degradation or loss of integrity. Density centrifugation typically requires a large amount of starting material, specialized equipment, and ultracentrifugation, making the process time-consuming and tedious. Alternative methods such as fluorescence-activated organelle sorting (FAOS), free flow electrophoresis (FFE), and immune-isolation of endosomes have been described, but they also require special equipment and have their own advantages and disadvantages (1).

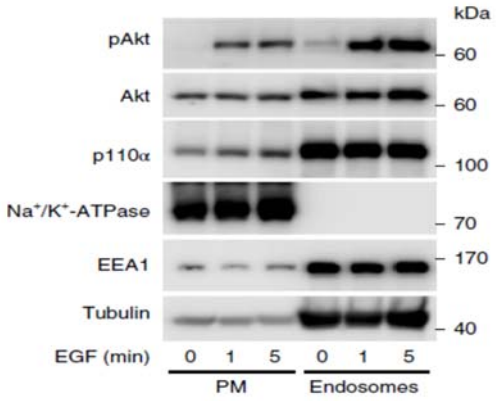
Introducing the Minute™ Endosome Isolation and Cell Fractionation Kit (Cat#: ED-028) from invent biotechnologies, a next-generation technology for analytical endosome isolation. This kit utilizes a spin column-based approach, enabling the separation of cells or tissues into endosomal and cytosolic fractions through differential centrifugation using only a tabletop centrifuge, followed by preferential precipitation. The isolated endosomes retain their native form and integrity, making them suitable for protein and nucleic acid analysis. The entire protocol can be completed in approximately 90 minutes. The efficacy of ED-028 has been consistently demonstrated in multiple studies, yielding robust and unambiguous outcomes.

In a study by Jin J. et al. (2), the activation of the nutrient-sensing molecule mTORC1 in T cells of older individuals was investigated. Naïve CD4+ T cells were activated for three days, followed by treatment with or without an AKT inhibitor before cell harvesting. Endosomes (E) were isolated and compared to the total cell lysate using Western blotting for different protein markers. As shown below, the endosomal compartment (EEA1) was significantly enriched compared to the total cell lysate, and the isolated endosomes were free from plasma membrane contamination (Na/K ATPase), confirming the excellent performance of ED-028.

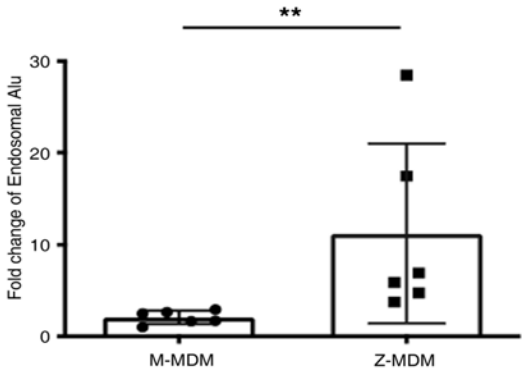



Thapa N. et al. (3) examined the spatial organization of phosphatidylinositol 3 kinase signaling at endosomal compartments using ED-028 in combination with a plasma membrane isolation kit from Invent

Biotechnologies (Cat# SM-005). MDA-MB-231 cells were collected for endosome and plasma membrane (PM) isolation, and the quantification of pAkt and Ptdins (p110a) was performed through Western blotting. As demonstrated, the endosomal compartment (EEA1) was significantly enriched compared to the PM fraction, while the PM fraction (Na/K ATPase) was free from contamination by the endosomal compartment.




In recent years, RNA therapeutics have emerged as promising treatments for human diseases. However, their large size, charge, and hydrophilicity prevent them from passively diffusing across the plasma membrane. Instead, they are taken up by cells through endocytosis and enter endosomes, where approximately 99% of the RNA therapeutics are captured and retained, with only 1% or less reaching the cytoplasm (4). The intact and native form of endosomes isolated by ED-028 allows for the study of RNA therapeutics and other RNA molecules using these isolated endosomes. Lee J. et al. (5) successfully isolated highly enriched endosome fractions from monocytes using ED-028. The isolated endosomes were then lysed, and endosomal RNAs, such as Alu RNA, were isolated and quantified using qPCR in control (M-MDM) and experimental (Z-MDM) samples.




Furthermore, ED-028 can also be employed in the study of viruses that enter cells through endocytosis. Drelich A. et al. investigated the mechanism of Ebola virus pathogenesis using isolated endosomes. The copy numbers of the virus were successfully determined using qPCR (6). While Western blotting is commonly used to analyze proteins in isolated endosomes, LC-MS/MS has also proven effective in analyzing the lipid compositions of endosomes isolated by ED-028 (7).

To summarize, density gradient centrifugation and other methods are valuable tools for endosome isolation but require careful optimization and consideration of potential pitfalls. These protocols are typically time-consuming and tedious. Our product offers a simple and rapid technology for isolating endosomes from cultured cells and tissues. As demonstrated by the aforementioned studies, the results obtained using ED-

028 are clear-cut, consistent, and reproducible. The isolated endosomes can be utilized for protein, nucleic acid, lipidomic, and other analyses.

**References**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