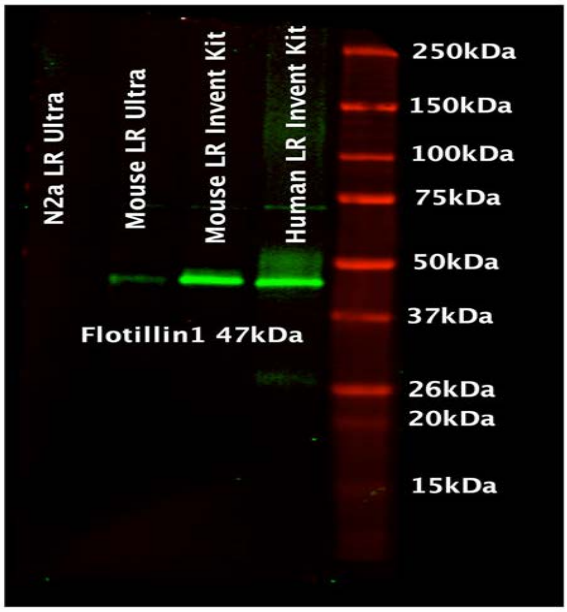
Lipid Raft Isolation Without Ultracentrifugation

(Invent Biotechnologies Inc.)

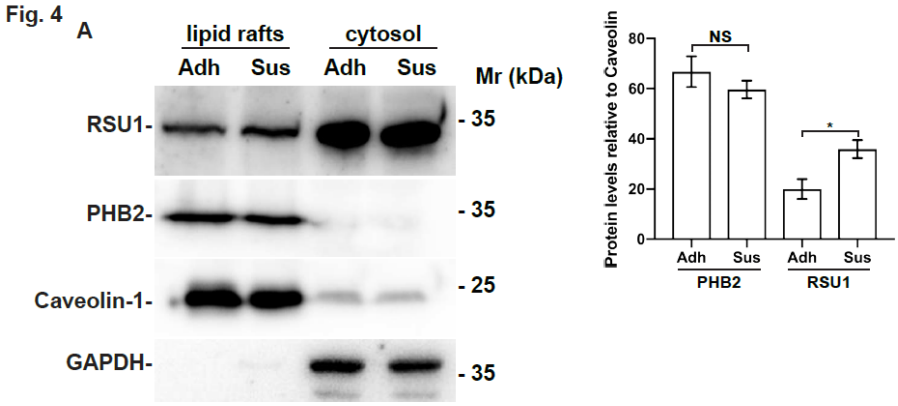
Lipid rafts are specialized membrane microdomains enriched in cholesterol and sphingolipids that play important roles in cellular processes such as signal transduction and membrane trafficking. Traditionally, lipid rafts have been defined by their insolubility in ice-cold Triton X-100 and low-buoyant density. These low-density membrane microdomains have been referred to as detergent-resistant membranes, Triton-insoluble membranes, and Triton-insoluble floating fraction. They are enriched in cholesterol, often sphingomyelin and various gangliosides. Isolation of lipid rafts is challenging due to their dynamic nature and low abundance, as well as the potential for co-isolation of non-raft components. Several methods have been developed to isolate lipid rafts, but each has its limitations and pitfalls. One of the most commonly used methods for lipid raft isolation is detergent extraction followed by sucrose gradient centrifugation using ultracentrifugation (membrane flotation). However, this method can result in non-specific extraction. The use of sucrose gradients can result in contamination of the raft fraction with other membrane components, as lipid rafts are not homogenous in their buoyant density. Another commonly used method is the use of specialized detergents such as Brij-98, followed by affinity purification with lipid raft markers such as caveolin-1 or flotillin-1 However, these markers are not exclusive to lipid rafts and can be found in non-raft regions of the membrane. low yield or low specificity are associated with affinity isolation protocols and may not be suitable for many experimental purposes (1, 2, 3,).

Lipid microdomains are mainly found in plasma membranes and similar structures also found in many organelles such as ER, Golgi apparatus and endosomes (4). Traditional methods for lipid raft isolation are for total lipid rafts only and specific isolation of lipid rafts from plasma membranes is not available. To address these issues, we have developed two lipid raft isolation kits (Cat# LR-039 and LR-042) based on our proprietary spin column-based technology without using ultracentrifugation. LR-039 is for isolation of total lipid rafts from cells/tissues and LR-042 is designed for isolation plasma membrane-derived lipid rafts. As shown below, these kits are not only simple and rapid to perform but they also superior to traditional ultracentrifugation method in terms of ease of use and purity.

Thorwald, M., A., et., al. (5) studied the pathogenesis of Alzheimer's disease (AD) using LR-039 to extract total lipid rafts from cortex samples and found that lipid rafts yield per gram AD frontal cortex was 20% below that of control. For validation purpose they also performed a side-by-side comparison of LR-039 with traditional ultracentrifugation method. The results are very convincing. Flotillin1 (a marker of lipid raft) is more abundant in lipid rafts isolated by LR-039 than that of ultracentrifugation for mouse and human samples. LR-039 extracted lipid rafts are void of cytosolic and nuclear contaminants.

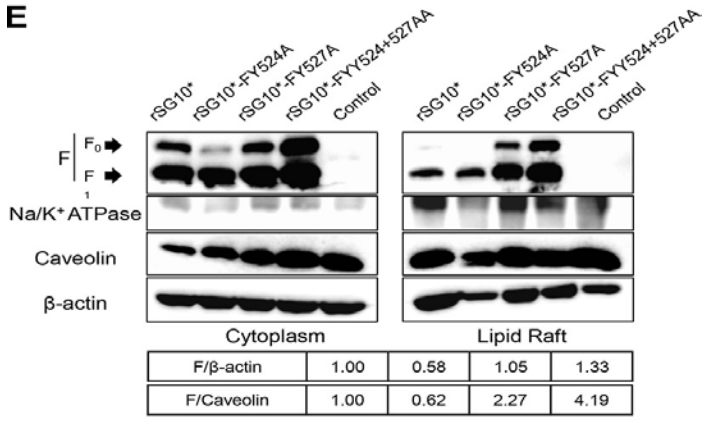


Excellent performance of LR-039 is also demonstrated by another study (6). In this study HT1080 cells that were adherent (Adh) or in suspension (Sus) were used for lipid rafts isolation. Extracted lipid rafts were probed with anti-RSU1, PHB2, Caveolin-1 and GAPDH in Western blotting. As show below (Fig.4), lipid raft marker caveolin-1 was significantly enriched in lipid raft fraction as compared to cytosolic fraction while GAPDH was only detected in cytosolic fraction. Significant relative expression of RSU1was only found in adherent cells. The clear-cut data and other results support that conclusion that focal adhesion protein RSU1 plays a critical role in cell-ECM detachment induced down-regulation of ERK signaling.



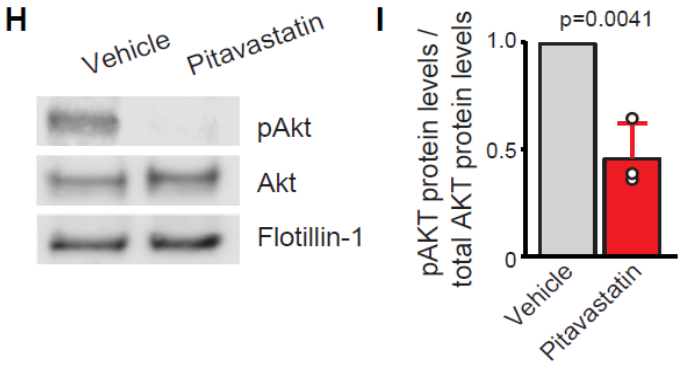
The cell membrane plays an important role in cellular function regulation, particularly signal transduction. Lipid rafts can selectively and dynamically recruit or exclude certain signaling proteins, kinases and phosphatases in response to stimuli inside and outside the cell by changing their size and composition and protecting related proteins from degradation, thus effectively promoting the interaction between proteins. It is therefore very important to isolate plasma membrane derived lipid rafts for the studies of cell signal transduction. We have developed a spin-column-based kit (cat# LR-042) for isolation of lipid rafts from plasma membrane. The excellent

performance of the kit is demonstrated in following figures. Bu, Y., et., al. (6) studied the localization of Newcastle disease virus F protein using viral infected BSR-T7/5 cells. Plasma membrane-derived lipid rafts were isolated using LR-042 and analyzed by Western blotting. As expected, strong expression of plasma membrane marker (Na/K-ATPase) and lipid raft marker (caveolin) are closely associated indication that the lipid rafts are indeed derived from plasma membrane. The expression patterns of F protein are also significantly different in cytoplasm and lipid rafts.



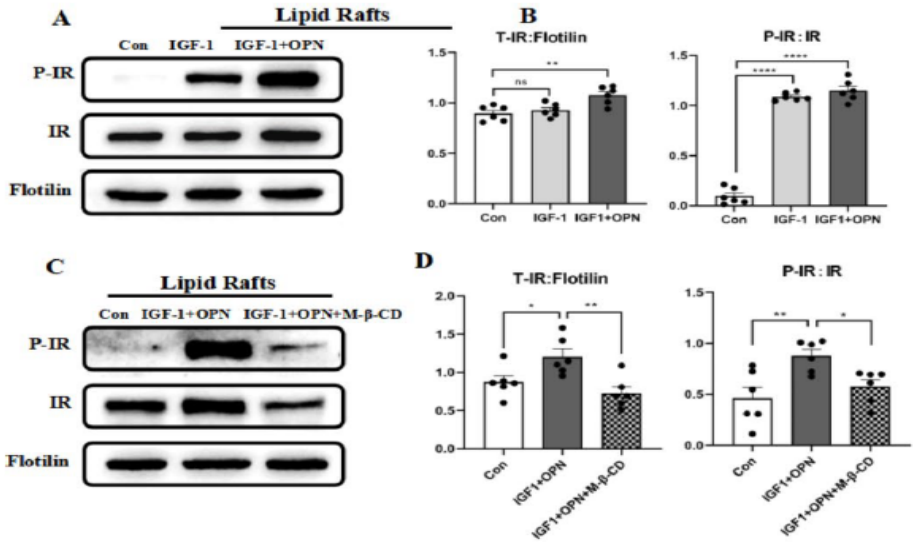
F/β-actin	1.00	0.58	1.05	1.33
F/Caveolin	1.00	0.62	2.27	4.19

In a study that investigated the pathogenesis and therapeutic mechanisms of acute lymphoblastic leukemias (7), lipid rafts were extracted from plasma membrane of CUTLL3 cells using LR-042, and analyzed for semi-quantification of phosphorylated Akt (pAkt). The results show that CUTLL3 cells treated with vehicle or Pitavastatin at an early timepoint (80% cell viability) causes marked decrease in pAkt while expression of lipid raft marker (flotillin) and non-phosphorylated Akt show no differences further supporting a role for Akt signaling as a key effector of antileukemic effects of statin in EPT-ALL cells. In this experiment, clean bands in Western blotting form the basis for accurate quantification of pAkt.



In another study aimed to investigate the effect of insulin-like growth factor 1(IGF-1) combined with osteopontin (OPN) on protein expression level and growth of neuronal axons and its mechanisms, Zhao, Q., et., al. (8) found that IGF-1combined with osteopontin promoted neuronal axon growth through the IGF-IR/Akt/mTOR signaling pathway in lipid rafts. In this study, plasma

membrane-derived lipid rafts were isolated from neurons (LR-042) and probed with IR, phosphorylated IR (P-IR) and flotillin (lipid raft marker) in Western blotting. As shown in the figure below, the combination of IGF-1 and OPN significantly increase the expression of P-IR without significant change in the expression flotillin in control and experimental groups indication the specificity of P-IR expression. Again, semi-quantification of expressed protein is possible only when protein bands are clear-cut with clean background.



In conclusion, the isolation of lipid rafts remains a challenging task, and the traditional approach is tedious and time consuming. Our spin column-based technology for lipid raft isolation is simple and straight forward. A table top centrifuge is the only equipment required, the whole protocol can be done in about 1h with consistent and reproducible results. The smaller sample size required is also a plus especially when the starting material is a limiting factor.

References:

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