Product Manual

Lipid Droplet Isolation Kit

Catalog Number

MET-5011 **50 preps**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Lipid droplets are organelles that are rich in lipids, contain a lipid rich core, and are surrounded by a phospholipid monolayer as well as outer lipid droplet associated proteins. Lipid droplets are commonly found in adipose tissue of animals, although they are found in all eukaryotes. Lipid droplets function to regulate the hydrolysis and storage of neutral lipids and also serve as storage for cholesterol and acyl-glycerols used to form and maintain cellular membranes.

Beyond the function of lipid and cholesterol storage, lipid droplet organelles have been more recently associated with inflammatory responses, obesity, atherosclerosis, and cancer. Lipid droplets have been shown to protect against lipotoxicity in non-adipocytes by storing fatty acids as neutral triacylglycerol. Lipid droplets also aid in protein binding and degradation and have been demonstrated to be used by pathogens such as dengue virus and hepatitis C Virus.

Cell Biolabs' Lipid Droplet Isolation Kit isolates lipid droplets by simple gradient centrifugation, but circumvents the need for large sample sizes or ultracentrifugation. A lipid droplet source such as tissue or cultured cells is homogenized. A gradient is then created with the homogenate, and the material is centrifuged. The lipid droplets float to the top of the gradient and are recovered by carefully pipetting from the top of the gradient. Each kit provides sufficient reagents to isolate up to 50 preps based on a 50-100 mg tissue or cultured cell sample size.

Related Products

1. STA-613: Lipid Quantification Kit (Colorimetric)
2. STA-612: Lipid Extraction Kit (Chloroform Free)
3. STA-330: TBARS Assay Kit (MDA Quantitation)
4. VPK-151: HCV Core Antigen Elisa
5. STA-617: Lipid Quantification Kit (Fluorometric)

Kit Components

1. Reagent A (Part No. 50111B): One 10 mL bottle.
2. 10X Reagent B (Part No. 50112A): One 7 mL bottle.

Materials Not Supplied

1. 15 mL conical polypropylene tubes
2. 2 mL microcentrifuge tubes
3. 27-gauge needles
4. 3 mL syringes
5. Glass dounce or other device for tissue homogenization

Storage

Store the entire kit at 4°C. To avoid possible leakage store bottles upright.

Preparation of Reagents

- 1X Reagent B: Dilute 10X Reagent B to 1X with deionized water. Stir to homogeneity.

Protocol

I. Isolation from Cultured Cells

1. Trypsinize 1.5-3 x 10^7 cells (roughly 50-100 mg) and resuspend in 10 mL of growth media in a 15 mL polypropylene tube.
2. Pellet cells at 1000 x g for 5 minutes.
3. Aspirate the media and wash cells with 10 mL of 1X PBS.
4. Pellet cells again at 1000 x g for 5 minutes.
5. Aspirate media and add 1 mL of 1X PBS.
6. Resuspend cells thoroughly and transfer to a 2 mL microcentrifuge tube.
7. Pellet cells again at 1000 x g for 5 minutes.
8. Aspirate 1X PBS and resuspend pellet thoroughly with 200 µL of Reagent A.
9. Incubate on ice for 10 minutes.
10. Add 800 µL of 1X Reagent B and mix well.
11. Incubate on ice for 10 minutes.
12. Homogenize the cells by passing them five times through a one inch 27-gauge needle attached to a 3 mL syringe.
13. Briefly spin the homogenate at 100 x g for 5 seconds
14. Carefully layer 600 µL of 1X Reagent B on top of the homogenate by dropwise addition taking care not to disturb the homogenate.
15. Spin the 2 mL microcentrifuge tube in a microcentrifuge for 3 hours at 18000-20000 x g at 4°C.
16. Carefully remove 270 µL (containing the floating lipid droplets) from the top of the tube and transfer to a fresh microcentrifuge tube.
17. Store lipid droplets at -80°C.

II. Isolation from Tissue by Dounce Homogenization

1. Weigh out 50-100 mg of tissue and mince into small pieces with a scalpel or scissors.
2. Transfer minced tissue to a glass dounce.
3. Add 200 µL of Reagent A.
4. Incubate on ice for 10 minutes.
5. Add 800 µL of 1X Reagent B.
6. Incubate on ice for 10 minutes.
7. Homogenize the tissue by performing 5 up and down strokes with the loose (A) pestle followed by 5 up and down strokes with the tight (B) pestle.
8. Transfer 1 mL of the homogenate to a 2 mL microcentrifuge tube

9. Carefully layer 600 µL of 1X Reagent B on top of the homogenate by dropwise addition taking care not to disturb the homogenate.
10. Spin the 2 mL microcentrifuge tube in a microcentrifuge for 3 hours at 18000-20000 xg at 4°C.
11. Carefully remove 270 µL (containing the floating lipid droplets) from the top of the tube and transfer to a fresh microcentrifuge tube.
12. Store lipid droplets at -80°C.

Example of Results

The following figures demonstrate typical results using samples prepared with the Lipid Droplet Isolation Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

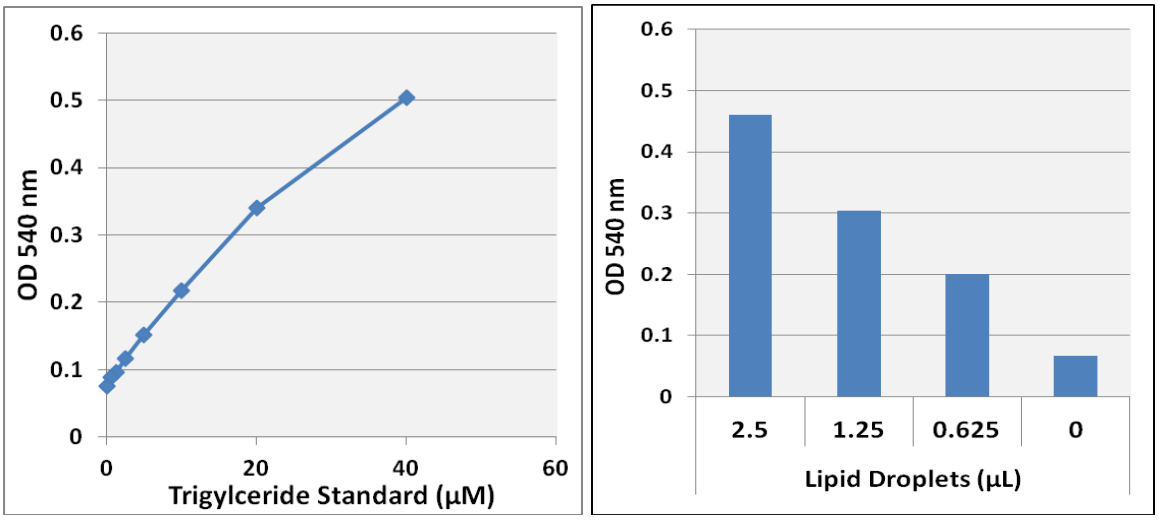

Figure 1: Triglyceride Quantification Kit (Colorimetric) (Cat. #STA-396) Performed on Extracted Lipids. (Left) Triglyceride Standard Curve. (Right) Lipid droplets isolated from Chicken Liver were tested for the presence of Triglyceride according to the Assay Protocol.

References

1. Thiam A.R., Farese R.V. Jr, and Walther T.C. (2013) *Nature Rev*. **14**, 775-786.
2. Martin S. and Parton R.G. (2006) *Nature Rev Mol. Cell Biol.***7**:373-378.
3. Greenberg, Andrew S.; Coleman, Rosalind A.; Kraemer, Fredric B.; McManaman, James L.; Obin, Martin S.; Puri, Vishwajeet; Yan, Qing-Wu; Miyoshi, Hideaki; Mashek, Douglas G. (2011). *Journal of Clinical Investigation* **121**: 2102–2110.
4. Brasaemle, D. L. (2007). *J. Lipid Res*. **48**: 2547–2559.
5. Bartz, R.; Li, W.-H.; Venables, B.; Zehmer, J. K.; Roth, M. R.; Welti, R.; Anderson, R. G. W.; Liu, P.; Chapman, K. D. (2007). *J. Lipid Res*. **48**: 837–847.

Recent Product Citations

1. Jung, J. et al. (2023). SB2301-mediated perturbation of membrane composition in lipid droplets induces lipophagy and lipid droplets ubiquitination. *Commun Biol*. **6**(1):300. doi: 10.1038/s42003-023-04682-9.

2. Hara, M. et al. (2023). PNPLA2 mobilizes retinyl esters from retinosomes and promotes the generation of 11-cis-retinal in the visual cycle. *Cell Rep*. **42**(2):112091. doi: 10.1016/j.celrep.2023.112091.
3. Chen, W.F. (2023). AmAtg2B-Mediated Lipophagy Regulates Lipolysis of Pupae in Apis mellifera. *Int J Mol Sci*. **24**(3):2096. doi: 10.3390/ijms24032096.
4. Soupene, E. & Kuypers, F.A. (2022). Dual Role of ACBD6 in the Acylation Remodeling of Lipids and Proteins. *Biomolecules*. **12**(12):1726. doi: 10.3390/biom12121726.
5. Kieu, T.L. et al. (2022). Downregulation of Elovl5 promotes breast cancer metastasis through a lipid-droplet accumulation-mediated induction of TGF-β receptors. *Cell Death Dis*. **13**(9):758. doi: 10.1038/s41419-022-05209-6.
6. Ohira, H. et al. (2022). Suppression of colonic oxidative stress caused by chronic ethanol administration and attenuation of ethanol-induced colitis and gut leakiness by oral administration of sesaminol in mice. *Food Funct*. doi: 10.1039/d1fo04120g.
7. Wu, S.C. et al. (2022). Stomatin modulates adipogenesis through the ERK pathway and regulates fatty acid uptake and lipid droplet growth. *Nat Commun*. **13**(1):4174. doi: 10.1038/s41467-022-31825-z.
8. Zhang, Z. et al. (2022). Loss of immunity-related GTPase GM4951 leads to nonalcoholic fatty liver disease without obesity. *Nat Commun*. **13**(1):4136. doi: 10.1038/s41467-022-31812-4.
9. Yuan, S. et al. (2021). SARS-CoV-2 exploits host DGAT and ADRP for efficient replication. *Cell Discov*. **7**(1):100. doi: 10.1038/s41421-021-00338-2.
10. Pu, Q. et al. (2021). Bitter receptor TAS2R138 facilitates lipid droplet degradation in neutrophils during Pseudomonas aeruginosa infection. *Signal Transduct Target Ther*. **6**(1):210. doi: 10.1038/s41392-021-00602-7.
11. Galano, M. et al. (2021). Role of Constitutive STAR in Leydig Cells. *Int J Mol Sci*. **22**(4):2021. doi: 10.3390/ijms22042021.
12. Ohira, H. et al. (2021). Alteration of oxidative-stress and related marker levels in mouse colonic tissues and fecal microbiota structures with chronic ethanol administration: Implications for the pathogenesis of ethanol-related colorectal cancer. *PLoS One*. **16**(2):e0246580. doi: 10.1371/journal.pone.0246580.
13. Du, X. et al. (2019). ORP5 localizes to ER-lipid droplet contacts and regulates the level of PI(4)P on lipid droplets. *J Cell Biol*. pii: jcb.201905162. doi: 10.1083/jcb.201905162.
14. Sugihara, M. et al. (2019). The AAA+ ATPase/ubiquitin ligase mysterin stabilizes cytoplasmic lipid droplets. *J Cell Biol*. **218**(3):949-960. doi: 10.1083/jcb.201712120.

Warranty

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