Version 1a Last updated 5 October 2020

ab242290 Lipid Droplet Isolation Kit

For the isolation of lipid droplets from tissue and cell homogenates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Lipid Droplet Isolation Kit (ab242290) 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.

The lipid droplets float to the top, but are not discernable from the soluble layer. They are recovered by careful 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.

2. Protocol Summary

Prepare tissue or cell homogenates.

Add Reagent A and incubate on ice for 10 mins.

Add Reagent B and incubate on ice for 10 mins.

Homogenize tissue or cells and spin down.

Layer Reagent B on top of homogenate and spin down for 3 hours at 18,000-20,000 x g at 4°C.

Remove lipid droplets and transfer to a fresh microcentrifuge, store at -80°C.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Reagent A	10 mL	4°C
10X Reagent B	7 mL	4°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 15 mL conical polypropylene tubes
- 2 mL microcentrifuge tubes
- 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- Microcentrifuge
- Tube vortexer
- 27-gauge needles
- 3 mL syringes
- Glass dounce or other device for tissue homogenization

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 10X Reagent B

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

7. Assay Procedure

7.1 Isolation from cultured cells:

7.1.1 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.
7.1.2 Pellet cells at 1000 *x g* for 5 minutes.
7.1.3 Aspirate the media and wash cells with 10 mL of 1X PBS.
7.1.4 Pellet cells again at 1000 *x g* for 5 mins.
7.1.5 Aspirate media and add 1 mL of 1X PBS.
7.1.6 Resuspend cells thoroughly and transfer to a 2 mL microcentrifuge tube.
7.1.7 Pellet cells again at 1000 *x g* for 5 mins.
7.1.8 Aspirate 1X PBS and resuspend pellet thoroughly with 200 µL of Reagent A.
7.1.9 Incubate on ice for 10 mins.
7.1.10 Add 800 µL of 1X Reagent B and mix well.
7.1.11 Incubate on ice for 10 mins.
7.1.12 Homogenize the cells by passing them five times through a one inch 27-gauge needle attached to a 3 mL syringe.
7.1.13 Briefly spin the homogenate at 100 *x g* for 5 secs.
7.1.14 Carefully layer 600 µL of 1X Reagent B on top of the homogenate by dropwise addition taking care not to disturb the homogenate.
7.1.15 Spin the 2 mL microcentrifuge tube in a microcentrifuge for 3 hours at 18,000 – 20,000 *x g* at 4°C.
7.1.16 Carefully remove 270 µL (containing the floating lipid droplets) from the top of the tube and transfer to a fresh microcentrifuge tube.
7.1.17 Store lipid droplets at -80°C.

7.2 Isolation from tissue:

7.2.1 Weigh out 50-100 mg of tissue and mince into small pieces with a scalpel or scissors.
7.2.2 Transfer minced tissue to a glass dounce.
7.2.3 Add 200 µL of Reagent A.
7.2.4 Incubate on ice for 10 mins.
7.2.5 Add 800 µL of 1X Reagent B.
7.2.6 Incubate on ice for 10 mins.
7.2.7 Homogenize the tissue by performing 5 up and down strokes with the loose pestle followed by 5 up and down strokes with the tight pestle.
7.2.8 Transfer 1 mL of the homogenate to a 2 mL microcentrifuge tube.
7.2.9 Carefully layer 600 µL of 1X Reagent B on top of the homogenate by dropwise addition taking care not to disturb the homogenate.
7.2.10 Spin the 2 mL microcentrifuge tube in a microcentrifuge for 3 hours at 18,000 – 20,000 *x g* at 4°C.
7.2.11 Carefully remove 270 µL (containing the floating lipid droplets) from the top of the tube and transfer to a fresh microcentrifuge tube.
7.2.12 Store lipid droplets at -80°C.

8. Typical Data

The following figure demonstrates typical results with Lipid Droplet Isolation Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

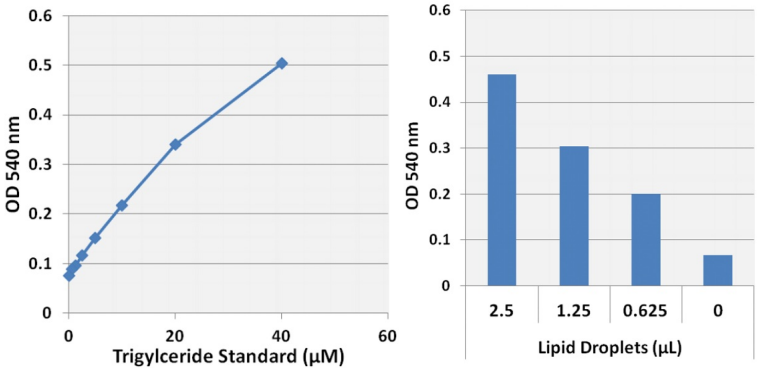

Figure 1. Extracted Lipids were analyzed using a Triglyceride Quantification Kit (Colorimetric). (Left) Triglyceride Standard Curve. (Right) Lipid droplets isolated from Chicken Liver were tested for the presence of Triglyceride according to the Assay Protocol.

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)