ab284556 – Hepatic Steatosis Assay Kit

For evaluating hepatic steatosis, quantifying triglycerides, and visualizing lipid droplet accumulation in Liver cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284556

Storage and Stability

On receipt entire assay kit should be stored at -20°C, except for PBS, Formalin, Oil Red O, and Methyl Green which should be stored at room temperature. Store all components protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Chloroquine	120 µL	-20°C
Formalin (10%)	24 mL	Room Temperature
Lipase	1 vial	-20°C
Methyl Green	24 mL	Room Temperature
Oil Red O	60 mg	Room Temperature
PBS	48 mL	Room Temperature
Triglyceride Assay Buffer	25 mL	-20°C
Triglyceride Enzyme Mix	1 vial	-20°C
Triglyceride Probe (in DMSO)	200 µL	-20°C
Triglyceride Standard (1 mM)	300 µL	-20°C

Materials Required, Not Supplied

- HepG2 cells (or other liver cells), can be purchased from ATCC
- 96-well white plate (fluorometric) or clear plate (colorimetric)
- Multi-well spectrometer (absorbance/fluorescence ELISA reader)
- Syringe and 0.2 µm syringe filter
- Light microscope
- Reagent grade isopropanol

Reagent Preparation

- Before using the kit, spin the tubes prior to opening. Components are stable for at least three months.

Triglyceride Assay Buffer, PBS, Formalin, and Methyl Green: Warm to room temperature before use.

Triglyceride probe: Ready to use as supplied. Protect from light. Warm to room temperature before use. Store at -20°C.

Lipase: Dissolve in 220 µL Triglyceride Assay Buffer. Aliquot and store at -20°C. Use within two months.

Triglyceride Enzyme Mix: Dissolve in 220 µL Triglyceride Assay Buffer. Aliquot and store at -20°C. Use within two months.

Triglyceride Standard: The Triglyceride Standard may separate during storage. To redissolve, keep the cap tightly closed, place in a hot water bath (>80°C) for 1 min. or until the standard looks cloudy and vortex for 30 sec. The Standard should become clear. Repeat heat and vortex one more time. The Triglyceride Standard is now in solution and ready to use.

Chloroquine: Warm to room temperature before use. Store at -20°C.

Oil Red O: Dissolve Oil Red O in 20 mL of 100% isopropanol to make a stock solution, mix well, and let it sit for 20 min. The stock solution is stable for one year. To make a working solution, add 3 parts of Oil Red O Stock Solution to 2 parts of dH2O, mix well, and allow it to sit for 10 min. Filter with 0.2 µm syringe filter. Prepare 15 min. before use. Working solution is stable for 2 hr.

Assay Protocol

Sample preparation:

1. Grow liver cells, e.g. HepG2, until confluent.
2. Add 1 mL 5% NP-40 per ~2 x 10^6 cells and collect cells using a cell scraper.
3. Homogenize cells and then heat the samples to 80-100°C for 2 min until the NP-40 becomes cloudy.
4. Vortex and cool to room temperature.
5. Repeat the heat/vortex/cool step one more time to solubilize all triglycerides.
6. Centrifuge for 2 min at 10,000 X g to remove insoluble material.
7. Collect supernatant.
8. For positive control, add 5 µL of Chloroquine per ml of media (= 25 µM) and filter through a 0.2 µm syringe filter under sterile conditions.
9. Treat HepG2 cells with media containing 25 µM Chloroquine for 3 days.
10. Homogenize cells as described above.
11. Add 2-50 µL test samples and positive control into a 96-well plate. Adjust the volume to 50 µL/well with Triglyceride Assay Buffer.

Δ Notes:
a) *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*
b) *Prepare parallel sample well(s) as sample background control(s) and adjust the volume to 50 µL/well with Triglyceride Assay Buffer.*
c) *Endogenous compounds in the sample may interfere with the assay, for accurate determination of Triglyceride in your sample; we suggest spiking parallel samples with a known amount of Triglyceride Standard (4 nmol for color and 0.4 nmol for fluorescence).*

Standard Curve Preparation

1. For colorimetric assay, prepare 0.2 mM Triglyceride Standard by adding 40 µL of 1 mM Triglyceride Standard to 160 µL Triglyceride Assay Buffer.
2. Mix well; add 0, 10, 20, 30, 40, and 50 µl of 0.2 mM Triglyceride Standard into a series of wells in 96-well plate.
3. Adjust the volume to 50 µL/well with Triglyceride Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of Triglyceride Standard.
4. For fluorometric assay, dilute the Triglyceride Standard further to 0.02 mM by adding 10 µL of 0.2 mM Triglyceride Standard to 90 µL Triglyceride Assay Buffer.
5. Mix well and use 0.02 mM Triglyceride Standard to generate the fluorometric Standard Curve by following the procedure mentioned for the colorimetric assay

Δ Note: *Detection sensitivity is 10-100 fold higher for a fluorometric assay than a colorimetric assay.*

Lipase

1. Add 2 µL of Lipase to each Standard, sample and positive control well and 2 µL Assay buffer to sample background control well(s).
2. Mix and incubate for 20 min at room temperature to hydrolyze triglyceride.

Triglyceride Reaction Mix

1. Mix enough reagent for the number of assays to be performed. Prepare 50 µL of Reaction Mix per well:

	Reaction Mix
Triglyceride Assay Buffer	46 µL
Triglyceride Probe*	2 µL
Triglyceride Enzyme Mix	2 µL

2. Add 50 µL of Reaction Mix to each well containing Standard, sample, positive control, or background control.
3. Mix well. Protect from light and incubate at room temperature for 30-60 min.

*For the fluorometric assay, use 0.4 µL/well of the Probe to decrease background reading and adjust the volume of Triglyceride Assay Buffer accordingly.

Measurement

Measure absorbance at 570 nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm for fluorometric assay in a microtiter plate reader. The reaction is stable for at least two hrs.

Calculation

1. Subtract 0 Standard reading from all readings.
2. If sample background control reading is significant then subtract the sample background control reading from sample reading.
3. Plot the Triglyceride (TG) Standard Curve.
4. For unspiked samples, apply the corrected OD to the Triglyceride Standard Curve to get B nmol of Triglyceride in the sample well.

Sample TG concentration (C) = B/V X D nmol/µL or mM

Where: **B =** amount of TG in sample well from Standard Curve (nmol)
V = sample volume added into the reaction well (µL)
D = sample dilution factor

Δ Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, TG amount in sample well (B) =

$$\left(\frac{OD\ sample(corrected)}{(OD\ sample + TG\ Std(corrected)) - (OD\ sample(corrected))}\right) * TG\ Spike\ (nmol)$$

Technical Support

For all technical or commercial enquiries please go to:

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