ab65336 – Triglyceride Assay Kit

For the rapid, sensitive and accurate measurement of triglycerides in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab65336 (use http://www.abcam.cn/ab65336 for China, or http://www.abcam.co.jp/ab65336 for Japan)

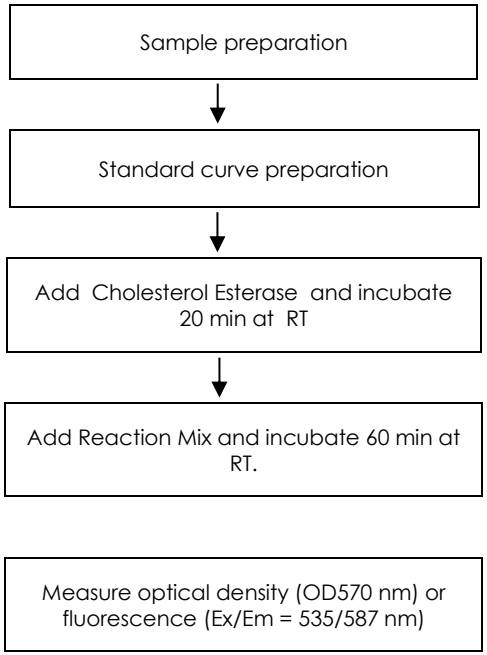
Background:

Triglyceride Assay Kit ab65336 is a no-wash assay that provides a sensitive, easy method to measure triglyceride concentration in a variety of samples including cells and tissue lysates, serum, heparin plasma and urine (UTI).

Triglycerides are converted to free fatty acids and glycerol. The glycerol is then oxidated to generate a product that reacts with a probe, generating a colorimetric (570 nm) and fluorometric (Ex/Em = 535/587 nm) readouts. The kit can detect 2 pmol – 10 nmol if triglycerides in a well. The kit also detects monoglycerides and diglycerides.

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Set plate reader to room temperature
- Solubilize Enzyme Mix VI, thaw OxiRed™ Probe, Triglyceride Standard and Wash Buffer I (aliquot if necessary); get equipment ready
- Prepare samples.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Set up plate for standard (50 µL) and samples (50 µL).
- Add 2 µL Cholesterol Esterase and incubate at RT for 20 min.
- Prepare Triglyceride Reaction Mix.
- Add 50 µL Reaction Mix to each well.
- Incubate plate at RT for 60 mins
- Measure plate at OD 570nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 5	25 mL	-20°C	-20°C.
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Cholesterol Esterase	1 vial	-20°C	-20°C
Enzyme Mix VI	1 vial	-20°C	-20°C
Triglyceride Standard	300 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labeled as Assay Buffer V and Triglyceride Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Triglyceride Probe. The composition has not changed.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- 96 well clear plate with flat bottom (colorimetric assay) / 96 well black plate with flat bottom (fluorometric assay)
- Orbital shaker
- Microcentrifuge
- Dounce homogenizer (if using tissue)
- NP-40 (Nonidet P-40) or Equivalent Non-Ionic Detergent (e.g. IGEPAL-CA630)
- 1 x Phosphate buffered saline (PBS), pH 7.4
- MilliQ water or other type of double distilled water (ddH2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Assay Buffer 5: Ready to use. Equilibrate to room temperature (RT).

Triglyceride Standard: Equilibrate to RT before proceeding. Storage at -20°C may cause the triglyceride standard to separate. To re-dissolve place in a hot water bath (~100°C) for at least 3 minutes or until the standard looks cloudy. Cool it down to RT & vortex for 30s.
***NOTE:** the standard should become clear.
Repeat the heat and vortex one more time. The Standard is now ready to use.
***NOTE:** The heating and mixing steps are critical to ensure the standard is fully dissolved and not producing low standard curve values. Each aliquot of standard should be boiled as described above before use.

OxiRed™ Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the frozen DMSO solution before use.

Enzyme Mix VI: Reconstitute in 220 µL Assay Buffer 5. Aliquot and store at -20°C. Keep on ice during the assay.

Cholesterol Esterase: Reconstitute in 220 µL Assay Buffer 5. Aliquot and store at -20°C. Keep on ice during the assay.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples at -20°C. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.
- NP-40 works better than Triton X-100 or Tween-20 to keep lipids in solution and does not create background for the assay.
- Less cells than recommended can be used, but the yield of triglycerides might be less. The number of cells needed will depend on the amount of triglycerides in them. If less cells are used the volume of NP-40/water can be scaled down proportionately.
- Sodium azide content above 0.05% and phenol red (if the color of the sample well is affected) can interfere with the assay.

Interferences:
- Glycerol in the samples could interfere with the assay, generating high background signals. Prepare a sample background controls to account for this interference.

Cells (adherent or suspension) samples:
1. Harvest the amount of cells necessary for each assay (initial recommend = 1 x 10^7 cells).
2. Wash cells with cold PBS.
3. Resuspend and homogenize samples in 1 mL of 5% NP-40/ddH_2O solution.
4. Slowly heat the samples to 80 – 100°C in a water bath for 2 – 5 minutes or until the NP-40 solution becomes cloudy, then cool down to RT.
5. Repeat previous step to solubilize all triglycerides
   ***NOTE**: If the lysed cells are not dissolving after 2 cycles of heating and cooling, the amount of 5% NP-40 in water can be increased, and the temperature can be raised.
6. Centrifuge for 2 minutes at top speed using a microcentrifuge to remove any insoluble material. Keep the supernatant and transfer to a new tube.
7. Dilute samples 10-fold with ddH2O before proceeding.

Tissue Samples:
1. Harvest the necessary amount of tissue for each assay (~100 mg of tissue).
2. Wash tissue with cold PBS.
3. Resuspend and homogenize samples in 1 mL of 5% NP-40/ddH2O solution using a Dounce homogenizer or pestle with 10 – 15 passes.
4. Slowly heat the samples to 80 – 100°C in a water bath for 2 – 5 minutes or until the NP-40 solution becomes cloudy, then cool down to RT.
5. Repeat previous step to solubilize all triglycerides.
   ***NOTE** If the lysed tissue is not dissolving after 2 cycles of heating and cooling, the amount of 5% NP-40 in water can be increased, and the temperature can be raised.
6. Centrifuge for 2 minutes at top speed using a microcentrifuge to remove any insoluble material.
7. Dilute samples 10-fold with ddH_2O before proceeding.

Serum and other Biological Samples: - Test directly.
Samples can be tested directly or diluted in assay buffer. To find the optimal values to ensure readings are withing the standard curve, we recommend using different volume of samples, or performing several dilutions of the sample.
***NOTE**: Serum typically contains 0.1 – 6 mM triglycerides

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

Prepare Triglyceride Standard as follows:

For Colorimetric Assay

Prepare a 0.2 mM Triglyceride Standard by diluting 100 µL of the 1 mM standard in 400 µL of Assay Buffer 5. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.

Using 0.2 mM Triglyceride Standard, add 0, 10, 20, 30, 40, 50 µl Triglyceride Standard into a series of wells, generating 0, 2, 4, 6, 8, 10 nmol/well of Triglyceride Standard. Adjust the volume to 50 µl/well with Assay Buffer 5. Or prepare triplicate standard curve dilutions as described below.

For Fluorometric assay

Prepare a 0.2 mM Triglyceride Standard by diluting 40 µL of the 1 mM standard in 160 µL of Assay Buffer 5. Dilute 50 µL of 0.2 mM standard with 450 µL of Assay Buffer 5 to give a 0.02 mM Triglyceride Standard. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.

Using 0.02 mM Triglyceride Standard, add 0, 10, 20, 30, 40, 50 ml Triglyceride Standard into a series of wells, generating 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of Triglyceride Standard. Adjust the volume to 50 µl/well with Assay Buffer 5. Or prepare duplicate standard curve dilutions as described below.

Using 0.2 mM (**colorimetric assay**) or 0.02 mM (**fluorometric assay**) Triglyceride Standard, prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.2 mM or 0.02 mM Standard (µL)*	Assay Buffer 5 (µL)	Final volume standard in well (µL)	End Triglyceride Amount (nmoles/well) Colorimetric Assay	End Triglyceride Amount (nmoles/well) Fluorometric Assay
1	0	150	50	0	0
2	30	120	50	2	0.2
3	60	90	50	4	0.4
4	90	60	50	6	0.6
5	120	30	50	8	0.8
6	150	0	50	10	1.0

***NOTE: For colorimetric assay use 0.2 mM standard, for fluorometric assay use 0.02 mM standard**

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.
- Endogenous compounds in the sample may interfere with the reaction so we recommend spiking samples with a known amount of standard (2 – 10 nmol) to ensure accurate determination of triglycerides in your sample.
- If you suspect your samples contain glycerol, set up Sample Background Controls to correct for background noise which may be caused by interference of glycerol with Cholesterol Esterase activity.

1. Set up Reaction wells:
   Standard wells = 50 µL standard dilutions.
   Sample wells = 2– 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).
   Background Control wells = 2-50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).
2. **Addition of Cholesterol Esterase:**
   Add 2 µL Cholesterol Esterase to Standard and Sample wells.
   Add 2 µL Assay Buffer 5 to Sample Background Control wells (do not add Cholesterol Esterase to these samples).
   Mix and incubate for 20 minutes at RT to convert triglyceride to glycerol and fatty acid. During this time ensure that the plate is under constant agitation.
3. **Triglyceride Reaction mix**
   Each well (standards, samples, and controls) requires 50 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:
   X µL component x (Number reactions +1).

Component	Colorimetric Assay Reaction Mix (µL)	Fluorometric Assay Reaction Mix (µL)
Assay Buffer 5	46	47.6
OxiRed™ Probe	2	0.4
Enzyme Mix VI	2	2

4. Mix Master Reaction Mix by inversion. Add 50 µL of the Master Reaction Mix to each well. Use a clean tip for each well.
5. Mix and incubate at RT for 60 minutes, protected from light.
6. Measure output immediately on a microplate reader at OD 570 nm for Colorimetric assay or Ex/Em= 535/587 nm for Fluorometric assay. The reaction is stable for at least 2 hours.

Calculations:

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

Non-spiked Samples:

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. If required, subtract the absorbance of the sample background control from the absorbance of the corresponding sample.
4. Plot the corrected absorbance values for each standard as a function of the final concentration of Triglyceride, and calculate the equation based on the corrected standard curve data using a linear regression.
5. Interpolate the amount of triglycerides in the sample wells (B) by using the linear equation, using the corrected readings for sample wells.
6. Concentration of triglyceride in nmol/µL (mM) in the test samples is calculated as:

$$Triglyceride\ concentration = \frac{B}{V} \times D$$

Where:
B = amount of triglyceride in the sample well calculated from standard curve (in nmoles).
V= sample volume added in the sample wells (in µL).
D= sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).
***NOTE** Triglyceride Standard molecular weight = 885.4 g/mol

Spiked Samples:

1. If using spiked samples, correct for any sample matrix interference by subtracting the sample reading from the spiked sample reading. This equation allows you to measure the triglyceride (TG) concentration in your sample when matrix interference is significant.

$$B = \left(\frac{(OD_{sample\ corrected})}{(OD_{spiked\ corrected}) - (OD_{sample\ corrected})}\right) * \text{TG Spike (nmol)}$$

Where:
B **=** TG amount in sample well (in nmoles)
OD $_{\text{sample corrected}}$ **=** OD/RFU of sample with background readings subtracted
OD $_{\text{spiked corrected}}$ **=** OD/RFU of spiked sample with background readings subtracted
TG Spike = amount of TG spiked (nmol) into the sample well

2. Calculate the triglyceride concentration using *B* and the equation above.

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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