

## Ab65336 – Triglyceride Assay Kit

For the measurement of triglycerides in various samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

**Storage and Stability:** Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze-thaws of reagents.

### Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer V/Triglyceride Assay Buffer	25 mL	-20°C	-20°C.
OxiRed Probe/Triglyceride Probe	200 µL	-20°C	-20°C
Cholesterol Esterase/Lipase	1 vial	-20°C	-20°C
Enzyme Mix VI/Triglyceride Enzyme Mix	1 vial	-20°C	-20°C
Triglyceride Standard/1 mM Triglyceride Standard	300 µL	-20°C	-20°C

### Materials Required, Not Supplied

Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)

Clear 96 well plate with flat bottom (for colorimetric assay) or black 96 well plate with flat bottom (for fluorometric assay)

Dounce homogenizer (if using tissue)

NP-40 (Nonidet P-40)

Phosphate buffered saline (PBS)

Plate shaker/orbital shaker

**Reagent Preparation:** Briefly centrifuge small vials at low speed prior to opening.

1. **Assay Buffer V/Triglyceride Assay Buffer:** Ready to use. Equilibrate to room temperature (RT).

2. **Triglyceride Standard (1 mM):** Equilibrate to RT before proceeding. Frozen storage 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.

**ΔNOTE:** the standard should become clear.

Repeat the heat and vortex one more time. The Standard is now ready to use.

**ΔNOTE:** 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.

3. **OxiRed Probe/Triglyceride 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.

4. **Enzyme Mix VI/Triglyceride Enzyme Mix:** Reconstitute in 220 µL Assay Buffer V/Triglyceride Assay Buffer. Keep on ice during the assay.

**Cholesterol Esterase/Lipase:** Reconstitute in 220 µL Assay Buffer V/Triglyceride Assay Buffer. Keep on ice during the assay.

**Standard Preparation** Always prepare a fresh set of standards for every use & discard working standard dilutions after use as they do not store well.

### 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 V/Assay Buffer.

### 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 V/Assay Buffer. Dilute 50 µL of 0.2 mM standard with 450 µL of Assay Buffer V/Assay Buffer to give a 0.02 mM Triglyceride Standard.

Prepare standard curve dilutions for either a colorimetric or fluorometric assay as described in the table in a microplate or microcentrifuge tubes. Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

Standard #	TG Standard (µL)	Assay Buffer V/Assay Buffer (µL)	Final volume standard in well (µL)	End amount TG standard (nmol/well)	
				Colorimetric	Fluorometric
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

### Sample Preparation

- Perform several dilutions of sample to ensure readings are within standard value range.
- Use fresh samples or snap freeze samples in liquid nitrogen upon extraction and store immediately at -80°C. When you are ready to test samples, thaw on ice. Note: this might affect the stability of samples, and 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

### Cells (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommend = 1 x 10<sup>7</sup> cells).

- Wash cells with cold PBS.
  - Resuspend and homogenize samples in 1 mL of 5% NP-40/ddH<sub>2</sub>O solution.
  - 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.
  - Repeat previous step to solubilize all triglycerides
- ΔNOTE:** 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.
- Centrifuge for 2 minutes at top speed using a microcentrifuge to remove any insoluble material. Keep the supernatant and transfer to a new tube.
  - Dilute samples 10-fold with ddH<sub>2</sub>O before proceeding.

#### Tissue Samples:

- Harvest the necessary amount of tissue for each assay (~100 mg of tissue).
  - Wash tissue with cold PBS.
  - Resuspend and homogenize samples in 1 mL of 5% NP-40/ddH<sub>2</sub>O solution using a Dounce homogenizer or pestle with 10 – 15 passes.
  - 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.
  - Repeat previous step to solubilize all triglycerides.
- ΔNOTE** 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.
- Centrifuge for 2 minutes at top speed using a microcentrifuge to remove any insoluble material.
  - Dilute samples 10-fold with ddH<sub>2</sub>O before proceeding.

#### Serum and other Biological Samples: - Test directly.

**ΔNOTE:** We recommend using different volumes of sample to ensure readings are within the standard curve range.

**ΔNOTE:** Serum contains 0.1 – 6 mM triglyceride

#### Assay Procedure

Equilibrate all materials and prepared reagents to RT just prior to use and gently agitate.

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).

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 lipase activity.

#### 1. Reaction wells set up:

Standard wells = 50 μL standard dilutions.

Sample wells = 2 - 50 μL samples (adjust volume to 50 μL/well with Assay Buffer V/Assay Buffer).

Sample Background Control wells = 2 - 50 μL samples (adjust volume to 50 μL/well with Assay Buffer V/Triglyceride Assay Buffer).

#### 2. Addition of Cholesterol Esterase/Lipase:

Add 2 μL Cholesterol Esterase/Lipase to Standard and Sample wells.

Add 2 μL Assay Buffer V/Triglyceride Assay Buffer to Sample Background Control wells (do not add Cholesterol Esterase/Lipase 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

- Prepare 50 μL of Triglyceride Reaction Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Colorimetric Reaction Mix (μL)	Fluorometric Reaction Mix (μL)
Assay Buffer V/Triglyceride Assay Buffer	46	47.6
OxiRed Probe/Triglyceride Probe	2	0.4
Enzyme Mix VI/Triglyceride Enzyme Mix	2	2

- Add 50 μL of Reaction Mix into each standard, sample, and background control wells.
- Mix and incubate at RT for 60 minutes protected from light.
- Measure output on a microplate reader at OD 570 nm for colorimetric assay or at Ex/Em = 535/587 nm for fluorometric assay.
- The reaction is stable for at least 2 hours.

#### Data analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

- Average the duplicate reading for each standard, control and sample.
- Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
- If significant, subtract the sample background control from sample readings.
- Plot the values for each standard as a function of the final concentration of triglyceride.
- Draw the best smooth curve through these points to construct the standard curve. Calculate the trendline equation based on your standard curve data.
- Apply the corrected sample OD reading to the standard curve to get triglyceride (B) amount in the sample wells.
- Concentration of triglyceride in nmol/μL (mM) in the test samples is calculated as:

$$\text{Triglyceride concentration} = \frac{B}{V} * D$$

#### Where:

**B** = amount of triglyceride in the sample well calculated from standard curve in nmol.

**V** = sample volume added in the sample wells (μL).

**D** = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

**ΔNOTE** Triglyceride Standard molecular weight = 885.4 g/mol

- 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.

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

#### Where:

**B** = TG amount in sample well (nmol)

**OD sample corrected** = OD/RFU of sample with blank and background readings subtracted

**OD spiked corrected** = OD/RFU of spiked sample with blank & background readings subtracted

**TG Spike** = amount of TG spiked (nmol) into the sample well

#### **Technical Support**

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