ab65336
Triglyceride Quantification Assay Kit (Colorimetric/Fluorometric)

For the measurement of triglycerides in various samples.

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

Triglyceride Quantification Assay Kit (Colorimetric/Fluorometric) (ab65336) is a sensitive and easy-to-use kit to measure triglyceride concentration in a variety of samples. In this assay, triglycerides are converted to free fatty acids and glycerol. The glycerol is then oxidized to generate a product which reacts with the probe to generate color (λ = 570 nm) and fluorescence (Ex/Em = 535/587 nm).

This assay can detect 2 pmol – 10 nmol (or 2 µM – 10 mM range) of triglyceride in various biological samples. The kit also detects monoglycerides and diglycerides.

Standard curve preparation

↓

Sample preparation

↓

Add lipase and incubate at RT for 20 minutes

↓

Add reaction mix and incubate at RT for 60 minutes protected from light

↓

Measure optical density (OD 570 nm) or fluorescence (Ex/Em = 535/587 nm)
2. Quick Assay Procedure

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

- Solubilize Triglyceride Standard, thaw Triglyceride Probe and prepare enzyme mix and lipase (aliquot if necessary); get equipment ready.
- Prepare TG standard dilution for your desired detection method: colorimetric (2 – 10 nmol/well) or fluorometric (0.2 – 1 nmol/well).
- Prepare samples in optimal dilutions so that they fit within the standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL), and if appropriate, for sample background control wells (50 µL).
- Add 2 µL of Lipase to standard and samples wells or 2 µL of Assay Buffer to sample background control wells. Mix and incubate for 20 minutes at RT.
- Prepare a master mix for Triglyceride Reaction Mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric Reaction Mix (µL)</th>
<th>Fluorometric Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride Assay Buffer</td>
<td>46</td>
<td>47.6</td>
</tr>
<tr>
<td>Triglyceride Probe</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Triglyceride Enzyme Mix</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

- Add 50 µL Triglyceride Reaction Mix to standard, sample and sample background control wells.
- Incubate plate at RT for 60 minutes.
- Measure plate at OD 570 nm for colorimetric assay or at Ex/Em = 535/587 nm for fluorometric assay.
3. Materials Supplied and Storage

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-thaw of reagents.

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Triglyceride Probe (in DMSO, anhydrous)</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Lipase (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Triglyceride Enzyme Mix</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>1 mM Triglyceride Standard</td>
<td>300 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
4. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- 96 well plate with clear flat bottom (for colorimetric assay) or black 96 well plate with clear flat bottom (for fluorometric assay)
- Dounce homogenizer (if using tissue)
- NP-40 (Nonidet P-40)
- Phosphate buffered saline (PBS)
5. 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.
6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 Triglyceride Assay Buffer:
Ready to use as supplied. Equilibrate to room temperature before use.

6.2 Triglyceride Standard (1 mM):
Equilibrate to room temperature before proceeding. Frozen storage may cause the triglyceride standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed and place in a hot water bath (~80 - 100°C) for 1 minute or until the standard looks cloudy, and then vortex for 30 seconds; the standard should become clear. Repeat the heat and vortex one more time. The Triglyceride Standard is now completely in solution, and 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.

6.3 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.

6.4 Triglyceride Enzyme Mix:
Reconstitute in 220 μL Triglyceride Assay Buffer. Keep on ice during the assay.

6.5 Lipase:
Reconstitute in 220 μL Triglyceride Assay Buffer. Keep on ice during the assay.
7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 For colorimetric assay

1. Prepare a 0.2 mM Triglyceride Standard by diluting 100 µL of the 1 mM standard in 400 µL of Assay Buffer.
2. Using the 0.2 mM standard, prepare standard curve dilutions as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>TG Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount of TG standard in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>90</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>60</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>30</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>0</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
7.2 For fluorometric assay

**Note:** the fluorometric assay detection sensitivity is 10-100 fold higher than the colorimetric assay

1. Prepare a 0.2 mM Triglyceride Standard by diluting 40 µL of the 1 mM standard in 160 µL of Assay Buffer.
2. Prepare a 0.02 mM Triglyceride Standard by diluting 50 µL of the 0.2 mM standard with 450 µL of Assay Buffer.
3. Using the 0.02 mM standard, prepare standard curve dilutions as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>TG Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount of TG Standard in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>90</td>
<td>50</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>60</td>
<td>50</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>30</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>150</td>
<td>0</td>
<td>50</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
8. Sample Preparation

General sample information:
- 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 for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you 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. Avoid multiple freeze-thaws.
- 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.

8.1 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 room temperature.
5. Repeat previous step to solubilize all triglycerides.

\( \Delta \) 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 to the high end of the range.

6. Centrifuge for 2 minutes at top speed using a microcentrifuge to remove any insoluble material.
7. Dilute samples 10-fold with ddH₂O before proceeding.

8.2 Tissue Samples:
1. Harvest the amount of tissue necessary for each assay (initial recommend = 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 room temperature.
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 to the high end of the range.

6. Centrifuge for 2 minutes at top speed using a microcentrifuge to remove any insoluble material.
7. Dilute samples 10-fold with ddH₂O before proceeding.

8.3 Serum, Cell Culture Supernatant, and other Biological Samples:
1. Test directly.

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

△ Note: Serum contains 0.1 – 6 mM triglyceride
9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

△ Note: Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determinations of triglycerides in the sample, we recommend spiking samples with a known amount of standard (2 – 10 nmol).

△ Note: Glycerol present in cell or tissue extracts can interfere with the lipase activity and generate background in the assay. If you suspect your samples contain glycerol, set up Sample Background Controls to correct for this background noise.

9.1 Reaction wells set up:
- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 - 50 µL samples (adjust volume to 50 µL/well with Triglyceride Assay Buffer).
- Sample Background Control wells = 2 - 50 µL samples (adjust volume to 50 µL/well with Triglyceride Assay Buffer).

9.2 Addition of Lipase:
1. Add 2 µL Lipase to Standard and Sample wells.
2. Add 2 µL Triglyceride Assay Buffer to Sample Background Control wells (do not add Lipase to these samples).
3. Mix and incubate for 20 minutes at room temperature to convert triglyceride to glycerol and fatty acid.

9.3 Triglyceride Reaction mix
1. Prepare 50 µL of Triglyceride Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.
## Component | Colorimetric Reaction Mix (µL) | Fluorometric Reaction Mix (µL)
---|---|---
Triglyceride Assay Buffer | 46 | 47.6 |
Triglyceride Probe | 2 | 0.4 |
Triglyceride Enzyme Mix | 2 | 2 |

**Note:** For fluorometric readings, using 0.4 µL/well of the Triglyceride probe decreases the background readings and thus increases sensitivity.

2. Add 50 µL of Reaction Mix into each standard, sample, and background control wells.
3. Mix and incubate at room temperature for 60 minutes protected from light.
4. 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.
10. 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.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of triglyceride.
5. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
6. Apply the corrected sample OD reading to the standard curve to get triglyceride (B) amount in the sample wells.
7. Concentration of triglyceride in nmol/µL (mM) in the test samples is calculated as:

\[
\text{Triglyceride concentration} = \frac{B}{V} \times D
\]

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 in Step 9.1).

△ Note: Triglyceride molecular weight = 885.4 g/mol
8. 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.

\[
B = \left( \frac{(OD_{sample \ corrected})}{(OD_{spiked \ corrected}) - (OD_{sample \ corrected})} \right) \times TG\ Spike\ (nmol)
\]

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 and background readings subtracted
- TG Spike = amount of TG spiked (nmol) into the sample well
11. Typical Data

Data provided for demonstration purposes only.

Figure 1. Typical Triglyceride Standard calibration curve using colorimetric reading.

Figure 2. Fluorometric triglyceride standard curve: mean of duplicates (+/- SD) with background readings subtracted.
Figure 3. Triglyceride measured in cell culture lysates showing quantity (nmol) per million cells. Samples with the concentration of $10^7$ cells/mL were used. Samples were diluted 40-80 fold and measured fluorometrically.
12. Notes
Technical Support

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