MA-0110

Triglyceride Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Triglycerides, a triester of 3 fatty acids and glycerol, are the primary constituent of body fat in both animals and plants. The types of fatty acids found in triglycerides encompass virtually every known naturally occurring fatty acid. A variety of lipases release fatty acids, which are then used for energy production or other purposes. AkrivisBio's Triglyceride Assay provides a simple, sensitive measurement of triglycerides from a variety of biological samples. Triglyceride content can be determined using either absorbance with a λ_{max} of 570 nm, useful range 0–10 nmol per well of a 96-well plate or fluorescence with Excitation/Emission of 535/587 nm with a lower limit of sensitivity in the low (1–5) picomole range.

Assay Principle:

1 - Mono-, di and triglycerides are hydrolyzed by lipase, generating free fatty acids and free glycerol
2 - Glycerol is oxidized generating a stoichiometric amount of hydrogen peroxide
3 – Hydrogen peroxide and our ADHP probe serve as substrates for peroxidase which results in the formation of intense color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0110-A
ADHP Solution	200 µl	Red	MA-0110-B
Lipase	lyoph	Blue	MA-0110-C
Glycerol Oxidation Mix	lyoph	Green	MA-0110-D
Triglyceride Standard	0.3 ml	Yellow	MA-0110-E

User Supplied Materials:

0.1 – 0.5% Peroxide-free detergent solution.

Storage and Handling:

Store kit at -20°C. Bring all kit materials to room temperature before use. Centrifuge all vials briefly before opening to dislodge components which may be adhering to the cap.
Triglyceride Standard: may come out of solution when frozen. To redissolve, heat the vial hot (80-100°C) water until it becomes cloudy (2-3 min). Vortex while hot which will cool it, converting it back to a clear solution. Repeat heating/cooling one time. The Standard is now ready to use.
ADHP Solution: DMSO freezes just below room temperature. It must be brought to room temperature before use. Store at –20°C.
Glycerol Oxidation Mix: Dissolve in 220 µl Assay Buffer. Best to store it as a few aliquots at -20°C to avoid repeated freeze/thaw cycles.
Lipase: Dissolve in 220 µl Assay Buffer. Aliquot and store at -20°C.

Assay Protocol:

1. Standard Curve:

For an absorbance-based assay, add 40 µl of triglyceride standard to 160 µl of Assay Buffer. Apply 0 – 10 – 20 – 30 – 40 – 50 µl to a series of wells in a 96-well plate and bring the well volumes to 50 µl with Assay Buffer. The wells have 0 – 2 – 4 – 6 – 8 – 10 nmoles of triglyceride respectively.
For a fluorescence-based assay, add 10 µl of triglyceride standard to 490 µl of Assay Buffer. Apply 0 – 10 – 20 – 30 – 40 – 50 µl to a series of wells, giving 0 – 200 – 400 – 600 – 800 – 1000 pmoles of triglyceride respectively. Due to the sensitivity of the fluorescence assay, it is possible to use a standard curve in the range of 0 – 200 pmoles by diluting the standard further.

2. Sample Preparation:

Cells (~10^6) are collected and lysed using 100 µl peroxide-free detergent solution. Tissues (10 mg) are homogenized in 100 µl of detergent solution. The homogenate/cell lysate should be subjected to a heating/cooling cycle similar to the Standard above. Body fluids (serum, CSF, saliva, etc.) can be used directly. Due to the wide range of triglyceride concentrations in samples, a preliminary test should be run to see if the amount is within the range of the Standard curve. Background controls should be run for each unknown sample to correct for any endogenous glycerol which may be present.
Occasionally matrix effects present a higher background and shallower standard curve due to altered activity of the kit enzymes. In this case, it is best to add an internal standard of 2 to 4 nmole of the triglyceride standard to 1 of two paired test samples. The difference in OD between the two samples allows for a determination of the unknown triglyceride content as a ratio of the added standard.

3. Sample Hydrolysis:

Add 2 µl of lipase to each Standard and sample well. Do not add lipase to the Background Control wells. Allow hydrolysis to proceed for 20 – 30 minutes.

4. Glycerol Oxidation:

Prepare sufficient Glycerol Oxidation Mix for the number of wells to be measured, each well requires 50 µl.

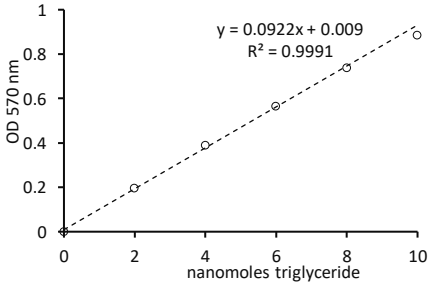
Reaction Mix:	absorbance	fluorescence
Assay Buffer:	46 µl	47.8 µl
ADHP Solution:	2 µl	0.2 µl
Glycerol Oxidation Mix:	2 µl	2 µl

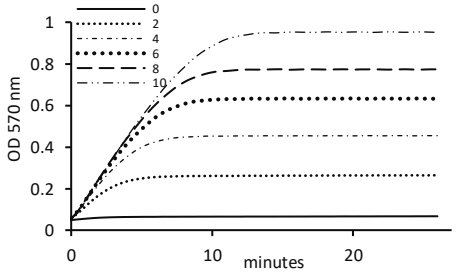
Add 50 µl of the Reaction Mix to each well containing the Triglyceride Standard, samples and Background Control. Mix well. Incubate at room temperature for 30-60 min. (60 min. gives slightly better result) protected from light. Alternatively, the reaction progress can be followed by monitoring the plate in a plate reader while the reaction proceeds. Being able to observe the reaction kinetics can frequently provide needed insight.

5. Measurement: Monitor the reaction progress with either absorbance (OD 570 nm) or fluorescence (Excitation/Emission = 535/587 nm). When the signal from the standards is no longer changing, the reaction is finished. It should take less than 60 minutes to reach endpoint.

6. Typical Results:

nmol Standard	Standard Raw Values OD	Background Corrected Values OD
0	0.0663	0
2	0.2634	0.1971
4	0.4543	0.388
6	0.6324	0.5661
8	0.8036	0.7373
10	0.9511	0.8848





48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com

7. Calculations: Subtract the 0 Standard reading from all standard readings. Plot the corrected absorbance or fluorescence standard curve. Determine the slope of the standard curve. The slope determines the OD/nmol or FL/pmol. There is a distinct convexity to the absorbance curve for values above about 1 OD. A similar convexity is also seen in fluorescence-based assays. Fitting the data to a 2nd order polynomial will give more accurate results with unknowns than a straight line.

Subtract the absorbance of the paired background controls from the absorbance of the test samples. Divide the background corrected sample readings by the slope of the Standard Curve to get nmoles in the well. To extrapolate back to triglyceride content of the original samples:

A. Divide the nmoles triglyceride in well by the volume of sample in µl added to the well = nmole triglyceride/µl sample

B. Multiply the nmole triglyceride/µl sample X µl total detergent solution added to sample = total nmol triglyceride in sample

C. Divide the total nmol triglyceride in sample by the mg tissue or (cell number (or mass) = nmol triglyceride/mg sample (of per cell number or mass)

Calculation for Internal standard used:

A. Absorbance $_{(\text{sample + added standard})}$ – Absorbance $_{(\text{sample only})}$ = Absorbance $_{(\text{internal standard})}$.

B. Absorbance $_{(\text{internal standard})}$ / nmoles standard added = OD/nmol triglyceride

C. Absorbance $_{(\text{sample only})}$ / OD/nmol triglyceride = Total nmol triglyceride in test sample.

D. Total nmol triglyceride in sample / [mg tissue (or cell number/mass) used] = nmol triglyceride per mg sample (or per cell number/mass)

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