ab155899

Free Glycerol Assay Kit II (Colorimetric)

Instructions for Use

For the sensitive and accurate measurement of free glycerol in various tissues/cells and for the analysis of metabolism and cell signaling.

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

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.

Version 4a, Last updated 21 August 2023

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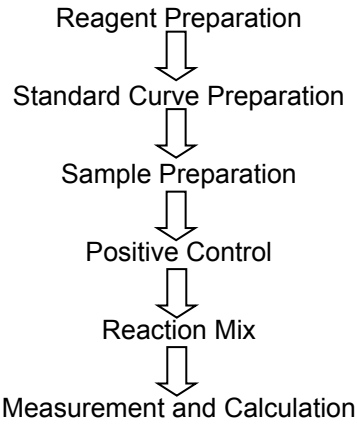
1. Overview

Glycerol is a central component of most lipids. It acts as a backbone for triglycerides and phospholipids, which play an important role in metabolism and cell membrane structure. Due to its low toxicity, glycerol is widely used in the pharmaceutical, food and cosmetic industries. Abcam's Free Glycerol Assay kit II is suitable for measuring free glycerol levels in samples that contain reducing substances, which may interfere with oxidase-based assays. In this assay, Glycerol in the presence of Glycerol Enzymatic Developer/Glycerol Enzyme Mix is converted to an intermediate, which reduces a colorless Developer Solution III/Probe to a colored product with strong absorbance at 450 nm. Free Glycerol Assay Kit II is simple, rapid and high-throughput adaptable. This assay kit can detect less than 20 µM of free Glycerol in various biological samples.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Glycerol Assay Buffer	25 mL
Glycerol Enzymatic Developer/Glycerol Enzyme Mix (lyophilized)	1 vial
Developer Solution III/Glycerol Probe (lyophilized)	1 vial
Glycerol Standard/Glycerol Standard (100mM)	0.2ml

* Store the kit at-20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all Buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

B. Additional Materials Required

- 96-well clear plate with flat bottoms

- Multi-well spectrophotometer (ELISA reader)

4. Assay Protocol

A. Reagent Preparation

1. Glycerol Enzymatic Developer/Glycerol Enzyme Mix:

Reconstitute with 220 µl Glycerol Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months. Keep on ice while in use.

2. Developer Solution III/Probe:

Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely. Store at -20°C. Use within two months.

3. Glycerol Standard:

Dilute Glycerol Standard to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM Glycerol Standard to 990 µl dH_2O. Mix well.

B. Glycerol Assay Protocol

1. Glycerol Standard Curve:

Add 0, 2, 4, 6, 8, 10 µl of 1 mM Glycerol Standard into a series of wells in 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well Glycerol Standard. Adjust volume to 50 µl/well with Glycerol Assay Buffer.

2. Sample Preparation:

Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Glycerol Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add 1-50 µl sample (2-100µg) per well, adjust final volume to 50 µl with Glycerol Assay Buffer.

Note:

a. NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control to subtract background from NADH.

b. Enzyme in some samples may interfere with the assay. Enzymes may be removed by using 10 kD spin column

c. For unknown samples, we suggest testing several doses of samples to ensure the readings are within the standard curve range.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix
Assay Buffer	46 µl	48 µl
Enzyme Mix	2 µl	---
Developer Solution III/Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples. Mix well.

Note: For samples having color or high NADH levels, add 50 µl of Background Control Mix to sample background control well(s). Mix well

4. Measurement:

Incubate for 60 min at 37°C and measure OD_{450nm}.

5. Data Analysis

Calculation: Subtract 0 Standard reading from all readings. Plot the Glycerol Standard Curve. Correct sample background by subtracting the value derived from the background control from sample readings. Apply the corrected sample reading to the Glycerol Standard Curve to get B nmol of Glycerol amount in the sample well(s).

$$\text{Sample Glycerol Concentration} = \frac{B}{V} \times \text{Dilution Factor} = \text{nmol/ml/ = µM}$$

Where:

B is the amount of Glycerol in the sample (nmol).

V is the sample volume used in the reaction well (ml).

Glycerol molecular weight: 92.09 g/mole.

Free glycerol in samples can also be expressed in nmol/mg of sample or any other desired method.

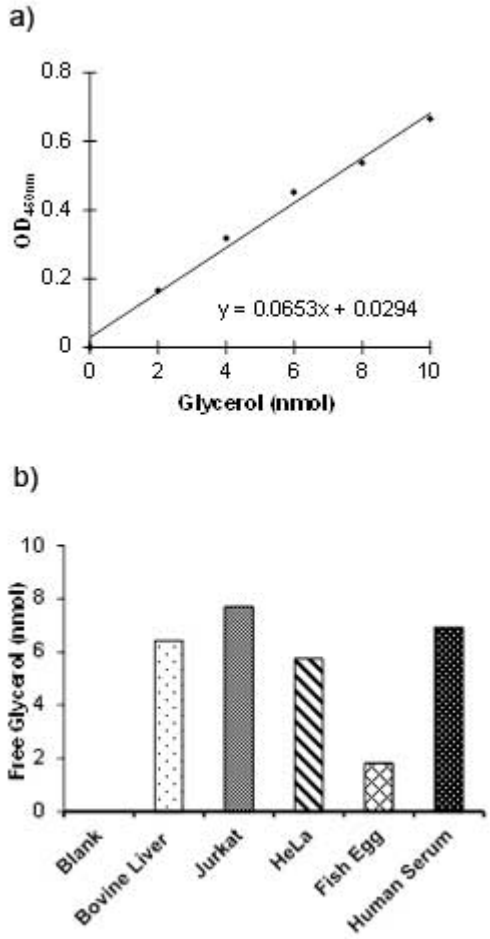


Figure 1: Glycerol Standard Curve [a]. Measurement of free glycerol in bovine liver (50 µg), Jurkat cells (40 µg), HeLa cells (30 µg), fish egg (58 µg) & human serum (2 µl) [b]. Assays were performed following kit protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

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