Glycerol Assay (GLY)

Cat. No. 8468
100 Tests in 96-well plate

Introduction

Glycerol is a precursor for synthesis of triglycerides and of phospholipids in the liver and adipose tissue. In some organisms, glycerol can be converted into glucose by the liver and provide energy for cellular metabolism. The measurements of circulating glycerol are considered to reflect lipolysis and are therefore useful parameters to evaluate in different conditions. This colorimetric assay is based on a coupled enzymatic reaction of glycerol, in which the formed hydrogen peroxide is catalyzed by peroxidase and reacts with 4-aminoantipyrine to form the product dye. The color intensity of the reaction product at 540nm is directly proportional to glycerol level in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8468a	1	Assay buffer	10 mL	4°C
8468b	1	Glycerol standard	0.1 mL	-20°C
8468c	1	Cofactor mix	0.2 mL	-20°C
8468d	1	Developer	1.6 mL	-20°C
8468e	1	Enzyme mix	0.3 mL	-20°C

Product Use

The Glycerol Assay kit measures the glycerol level of different types of samples, such as cell lysate, tissue extracts, serum and plasma. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from Glycerol Assay kit measuring glycerol solutions with concentrations ranging from 0.0625 to 2 mM show a linear relationship between OD_{540nm} and glycerol concentration (Figure 1). Linear detection range is 10 µM to 2 mM in 96-well plate assay.

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of glycerol standard

1. Add 1 µL of glycerol standard (8468b) to 249 µL of assay buffer (8468a) to make a 0.25 mL solution of 4mM glycerol.
2. Obtain 7 test tubes, add 25 µL of assay buffer (8468a) into each tube and label them #1 through #7.
3. Add 25 µL 4 mM glycerol into tube #1 and mix well to get the 2 mM glycerol standard.
4. Transfer 25 µL of the 2 mM glycerol standard from tube #1 to tube #2 and mix well to get the 1 mM glycerol standard.
5. Repeat step 4 for tubes #3-6 to serially dilute the glycerol standards. Do not add any glycerol to tube #7, which serves as blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each glycerol standard by aliquoting 10 µL/well of each glycerol standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	2 mM	1 mM	0.5 mM	0.25 mM	0.125 mM	0.0625 mM	Blank
B	2 mM	1 mM	0.5 mM	0.25 mM	0.125 mM	0.0625 mM	Blank

B. Preparation of test samples

1. Cell or tissues can be homogenized in 4 volumes of the assay buffer (8468a). Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 69 µL assay buffer (8468a), 2 µL cofactor mix (8468c), 16 µL developer (8468d) and 3 µL enzyme mix (8468e).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing glycerol standard, samples and blank. Measure the plate immediately at 540 nm with an ELISA plate reader to get A_1.
3. Incubate for 20 minutes at room temperature in dark. Read the absorbance at 540 nm with an ELISA plate reader to get A_2.

D. Calculations

1. Subtract the A_1 value from the A_2 obtained with all other standard and samples to get $\Delta A_{2\text{-}1}$value.
2. Subtract the $\Delta A_{2\text{-}1}$value of the blank from the $\Delta A_{2\text{-}1}$ value obtained with all other standard and samples to get $\Delta\Delta A_{2\text{-}1}$ value.
3. Based on the calibrated $\Delta\Delta A_{2\text{-}1}$ of the glycerol standard, make a standard curve by plotting $\Delta\Delta A_{2\text{-}1}$ as a function of glycerol concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.

4. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the glycerol concentration of test samples as follows:

$$[\text{Glycerol}] = \frac{\Delta\Delta A_{2\text{-}1} - B}{A}$$

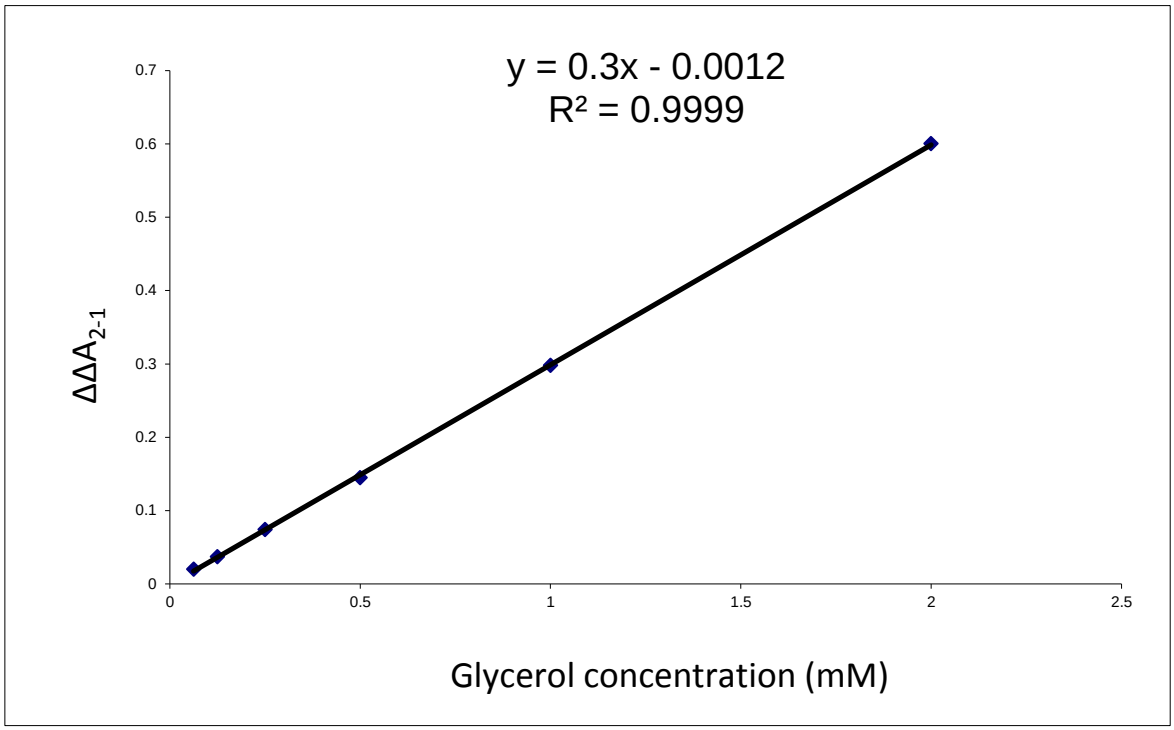

Figure1. A typical glycerol standard curve measured by ScienCell™ Glycerol Assay kit