Glycerol Assay Kit

Catalog No.: abx090674

Size: 96 tests

Detection Range: 0.01 mmol/L – 1.00 mmol/L

Sensitivity: 0.01 mmol/L

Storage: Store all components at 2-8°C in the dark.

Application: For detection and quantification of Glycerol in serum, plasma, tissue homogenates and cell lysates.

Introduction

Abbexa's Glycerol Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Glycerol concentration. Glycerol present in samples reacts to produce hydrogen peroxide, which reacts to produce a red compound that has an absorption maximum at 510 nm. The intensity of the red colour is proportional to the Glycerol content, which can then be calculated.

Kit components

1. 96-well microplate
2. Detection Reagent: 25 ml
3. Standard (1 mmol/L): 2 × 1 ml
4. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (510 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator
7. Vortex mixer
8. Isopropanol (Analytical Grade)

Protocol

A. Preparation of samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Collect the serum using a serum separator tube and allow to stand for 1-2 h at room temperature or overnight at 4°C. Centrifuge for 15 min at 2000 × g at 4°C. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.
- **Plasma**: Collect the plasma in a tube using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1000 × g at 2-8°C within 30 min of collection. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.
- **Tissue homogenates**: Weigh 0.02-1 g of tissue. For each 1 g of tissue, add 9 ml of Isopropanol. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication on ice. Incubate in a water bath at 70°C for 10 minutes. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately.
- **Cell lysates**: Collect 2×10^6 cells into a centrifuge tube and wash with PBS (0.01 M, pH 7.4) twice. Centrifuge at 1000 × g for 10 min and discard the supernatant. For every 2×10^6 cells, add 100 µl of isopropanol to the pellet. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication on ice. Incubate in a water bath at 70°C for 10 minutes. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately.

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.
- Samples should be free of hemolysis or turbidity.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples using Double Distilled Water and then carry out the assay procedure.

The recommended dilution factors are recommended for the following sample types (for reference only):

Sample Type	Dilution Factor
10% Mouse liver tissue homogenate	2
10% Mouse kidney tissue homogenate	1
10% Mouse spleen tissue homogenate	1
Cell lysates	1

B. Assay Procedure

1. Bring all reagents to room temperature before use. Set Standard and Sample wells on the 96 well microplate and label accordingly.
2. Prepare 8 tubes according to the following table.

Volume of 1 mmol/L Standard (µl)	Volume of Double Distilled water (µl)	Standard concentration (mmol/L)
0	200	0
20	180	0.1
40	160	0.2
80	120	0.4
100	100	0.5
120	80	0.6
160	40	0.8
200	0	1.0

3. Add 10 µl of each standard to the corresponding standard wells.
4. Add 10 µl of sample to the sample wells.
5. Add 250 µl of Detection Reagent to all wells.
6. Mix fully, and incubate at 37°C for 10 minutes.
7. Read and record the absorbance at 510 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the corrected OD value ($\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}}$) of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The concentration of the samples can be interpolated from the standard curve.

1. **Serum and plasma**

$$\text{Glycerol (mmol/L)} = \frac{\Delta \mathrm{A_{510}} - b}{\mathrm{a}} \times f$$

2. **Tissue homogenates**

$$\text{Glycerol (mmol/kg)} = \frac{\Delta \mathrm{A_{510}} - b}{\mathrm{a}} \times \frac{V}{m} \times f$$

3. **Cell lysates**

$$\text{Glycerol (µmol/10}^6\text{ cells)} = \frac{\Delta \mathrm{A_{510}} - b}{\mathrm{a}} \times \frac{V}{N} \times f$$

where:

a	Gradient of the standard curve ($y = ax + b$)
b	Intercept of the standard curve ($y = ax + b$)
$\mathrm{OD_{Standard}}$	Absorbance of each standard
$\mathrm{OD_{Blank}}$	Absorbance of the blank well (0 mmol/L)
$\mathrm{OD_{Sample}}$	Absorbance of each sample
$\Delta \mathrm{A_{510}}$	$\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}$
V	Volume of isopropanol (ml)
m	The wet weight of the sample (g)
N	The number of cells $\times 10^6$ (e.g. for 4×10^6 cells, N=4)
f	Sample dilution factor