



Glycerol

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1231

(Version 1.4C)

Detection and Quantification of Glycerol Content in Serum, Plasma,
Tissue extracts, Cell lysate, Cell culture media and Other biological
fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

In the food industry, glycerol is an important moistening agent for baked goods. It is also added to candies and icings to prevent crystallisation and as a solvent for food colours and a carrier for extracts and flavouring agents. As a product of fermentation, glycerol is monitored in the beer and wine industries, where it occurs at concentrations of approx.

GlycerolColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of Glycerol in a variety of samples. The glycerol is subsequently measured by a coupled enzymatic reaction system with a colorimetric readout at 550 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Diluent	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C
Standard (5 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.3125/0.156 mmol/L.

Enzyme: Add 9 ml Diluent to dissolve before use. Store at -20 °C for 2-3 weeks.

Dye Reagent: Add 10 ml Diluent to dissolve before use. Store at 4°C for 2-3 weeks or -20 °C for 2 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml distilled water for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml distilled water, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Distilled water	--	--	10 μ l
Enzyme	90 μ l	90 μ l	90 μ l
Dye Reagent	100 μ l	100 μ l	100 μ l
Cover the plate adhesive strip and put the plate into the oven, 37 °C for 10 minutes, record absorbance measured at 550 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{\text{Sample}} - OD_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\text{mmol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times C_{\text{Protein}} \times V_{\text{Sample}}} (\mu\text{mol/mg})$$

2.2 According to the quantity of cells

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.4 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

V_{Assay} : the volume Assay Buffer in sample preparation, μl

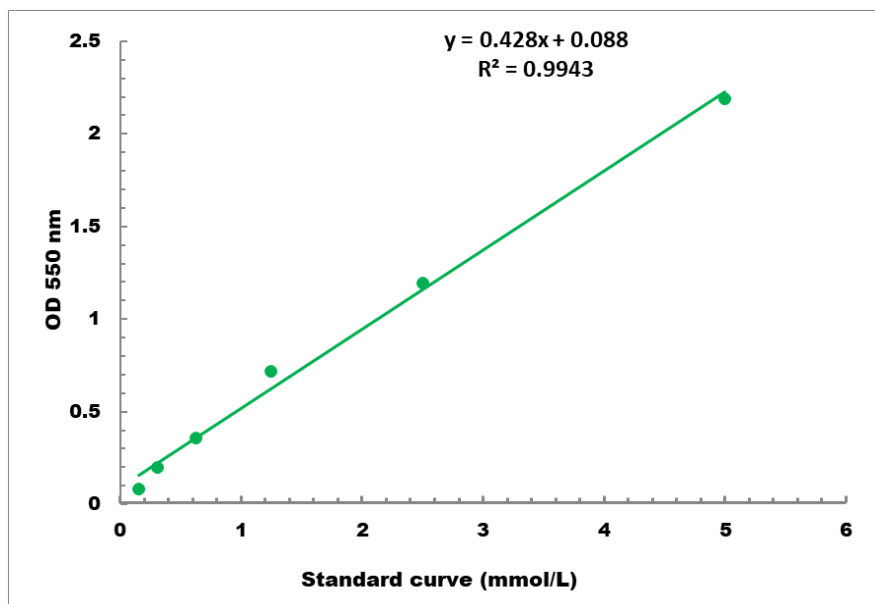
C_{Protein} : the sample protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell, 10^4

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 5 mmol/L