GAPDH Microplate Assay Kit

User Manual

Catalog # CAK1289

(Version 1.1A)

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

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

I. INTRODUCTION

GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase; EC 1.2.1.12) catalyzes the conversion of Glyceraldehyde-3-Phosphate (GAP) to 1, 3-Bisphosphate Glycerate (BPG) and plays a key role in glycolysis. The enzyme is involved in cellular processes such as apoptosis, membrane trafficking, iron metabolism and nuclear translocation. GAPDH (housekeeping gene) expression is stable and constitutive. Deregulation of GAPDH activity is associated with abnormal cell proliferation and carcinogenesis. Accurate quantitation of GAPDH activity is important for diagnosing diseases and studying normal cellular physiology.

GAPDH Microplate Assay Kit provides a simple and sensitive method for monitoring GAPDH activity in various samples. In this assay, GAPDH catalyzes conversion of GAP into BPG and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 492 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	30 µl x 1	-20 °C
Coenzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Reaction Buffer to dissolve before use; store at -20 °C for a month after reconstitution.

Coenzyme: add 1 ml Reaction Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

Dye Reagent A: add 9 ml distilled water to dissolve before use, mix; store at -20 °C for a month after reconstitution.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L; store at -80 °C for a month after reconstitution.

Positive Control: add 200 µl Assay Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 492 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 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 Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm the all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	70 µl	70 µl	--	--	70 µl
Coenzyme	10 µl	10 µl	--	--	10 µl
Substrate	10 µl	10 µl	--	--	10 µl
Sample	10 µl	--	--	--	--
Positive Control	--	--	--	--	10 µl
Standard	--	--	100 µl	--	--
Distilled water	--	10 µl	--	100 µl	--
Dye Reagent A	90 µl	90 µl	90 µl	90 µl	90 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, cover the plate adhesive strip, put the plate into the convection oven, incubate at 37 °C for 5 minutes, measured at 492 nm and record the absorbance.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: one unit of GAPDH activity is defined as the enzyme oxidize 1 μmol NADH per minute.

1. According to the protein concentration of sample

$$GAPDH\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GAPDH\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$GAPDH\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$GAPDH\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.8 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 0.4 μmol/ml;

$V_{Standard}$: the volume of standard, 100 μl = 0.1 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

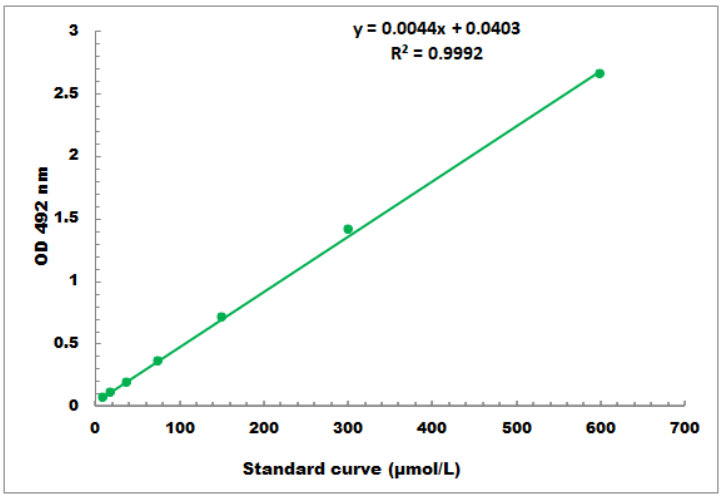
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

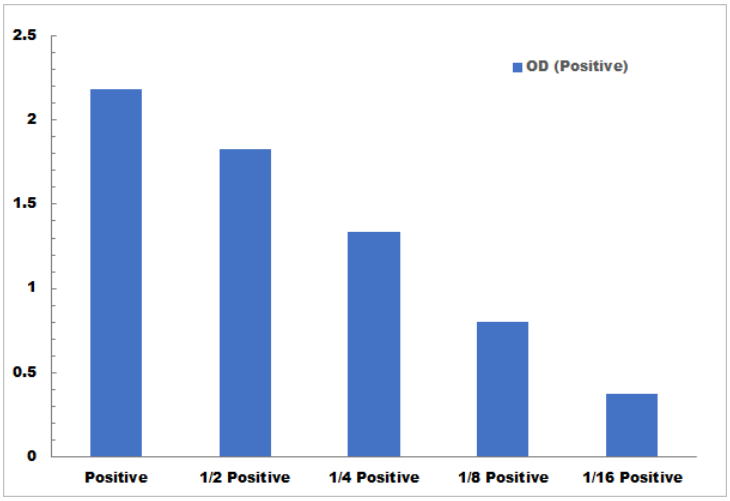
T: the reaction time, 5 minutes.

VII. TYPICAL DATA

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



Detection Range: 4 μmol/L - 400 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES