Tribioscience

Glucose 6-Phosphate Dehydrogenase Activity Colorimetric Assay

(TBS2102C, 100 Assays, Store at -20°C)

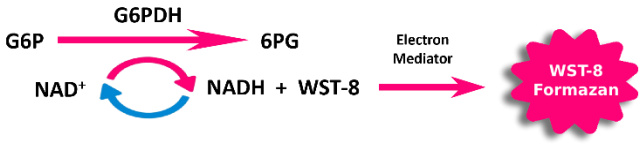
DESCRIPTION

Glucose 6-Phosphate dehydrogenase (G6PDH) is a key regulatory enzyme in the first step of the pentose phosphate pathway. In the presence of $NADP^+$ or NAD^+, it oxidizes glucose-6-phosphate (G6P) to 6-Phosphogluconolactone (6PG) and NADPH or NADH. It plays a vital role in numerous biosynthetic pathways and is important for preventing oxidative damage. In humans, deficiency is an autosomal hereditary disorder that can result in the depletion of NADPH in red blood cells and increases the risk of hemolytic anemia in situations of oxidative stress. On the other hand, inhibition of G6PDH has been linked to anti-cancer activity by decreasing RNA biosynthesis and increasing the buildup of reactive oxygen species in tumor cells.

Tribioscience's G6PDH Activity Colorimetric Assay kit provides a flexible, accurate, sensitive, and time-saving approach for detecting G6PDH activity in a wide variety of samples.

In this assay, G6PDH converts glucose-6-phosphate to 6-phosphogluconate and NAD(P)H, which in turn induces the Colorimetric Probe to generate an intense colored product at 460 nm as shown in Fig.1.

Fig.1. Principle of the Assay



FEATURES

- **Flexible:** Suitable for 96-well plate.
- **Accuracy**: Absorbance measurement is proportional to the G6PDH concentration.
- **Sensitive:** Can detect as low as 0.2 mU/mL.
- **Time-saving**: Just incubate and read out style.

Kit Components for 100 and 500 Assays, stored at -20°C.

Part Name	Part Size (100 tests)
G6PDH Substrate	60 µL
G6PDH Cofactor	20 µL
G6PDH Colorimetric Detection Probe	1.2 mL
G6PDH Assay Buffer	10 mL
G6PDH Positive Control (10X)	30 µL
NADH Standard (2 mM stock)	200 µL

APPLICATIONS

- Measure G6PDH activity in a variety of samples like cells and tissues.

DIRECTIONS FOR USE

Sample and positive control Preparation

1. Cells or Tissues are homogenized in 3 volumes of the assay Buffer.
2. Centrifuge the samples at 2000xg for 10 min at 4°C.
3. Take the supernatant to new tube on the ice. If not do the assay the same day, freeze the samples at -80°C for a month.
4. Do a 10X dilution of the positive control with the assay buffer: add 20 µL positive control to 180 µL assay buffer.

Assay Procedures

1. NADH Standard Preparation as Table below.

Tube #	Volume of NADH standard (µL)	Volume of Assay Buffer (µL)	NADH concen-tration (µM)	Total NADH (nmol/ well)
1	56.3	93.8	750	37.50
2	37.5	112.5	500	25.00
3	18.8	131.3	250	12.50
4	7.5	142.5	100	5.00
5	3.8	146.3	50	2.50
6	1.9	148.1	25	1.25
7	0	150	0	0.0

2. Load samples:
Add 50 µL of Standard, sample, and diluted positive control to each well. (Note: recommend running a pilot study to determine the optimal concentration of sample within the assay standard curve range).

3. Assay Working Reagent Preparation for 100 tests: please adjust the volume as you need.
Mixing the below reagent for 100 tests: total volume is 5 mL.

- 3940 µL Assay Buffer.
- 50 µL Substrate solution.
- 10 µL Cofactor Stock solution
- 1000 µL Detection Probe.

4. Add 50µL of above assay working reagent to each well containing the standards, diluted positive control, and the test samples.

5. Incubate at room temperature for about 20 ~ 60 minutes, with gentle shaking and protected from light.

6. Measure $OD_{460\ nm}$ in a kinetic model, choose two time points to measure (A_1 at T_1 and A_2 at T_2). The NADH standard can read in the end point.

7.Calculation:

Calculate the ΔA ($OD_{460\ nm}$) = A_2 - A_1

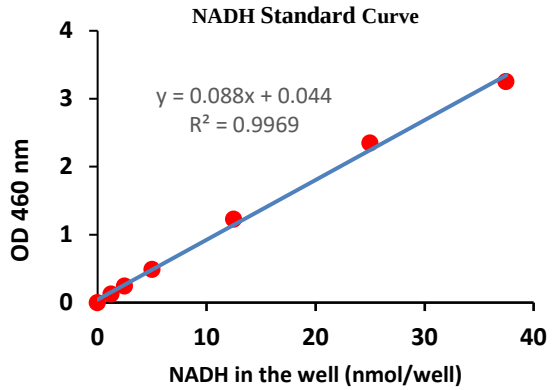
Apply the ΔA to the NADH standard curve to get B nmol of NADH generated by G6PDH during the reaction time (ΔT = T2 - T1).

V is the sample volume added into the reaction well (mL).

Sample G6PDH activity (mU/mL) = B/(ΔT x V) x Dilution Factor

Unit Definition: One unit of glucose 6-phosphate dehydrogenase (G6PDH) is the amount of enzyme that will generate 1.0 µmol of NADH per minute at room temperature using glucose-6-phosphate as the substrate.

Typical standard curve of NADH as shown in Fig2.



RELATED PRODUCTS

6-Phosphogluconate Colorimetric Assay (TBS2089C)
Glucose-6-phosphate dehydrogenase Activity Colorimetric Assay (TBS2102C)
ATP Colorimetric/ Fluorometric Assay (TBS 2010)
ADP Colorimetric/Fluorometric Assay (TBS3020)
Hexokinase Activity Colorimetric Assay (TBS2107C)
Glucose-6-Phosphate Dehydrogenase Activity Colorimetric Assay (TBS2102C)
Glucose-6-Phosphate Colorimetric Assay (TBS2103C)

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