

SPECIFICATION SHEET

Product Name: Glucose-6-Phosphate Dehydrogenase
Catalog # GD03D-E311H

Product Description: Recombinant research grade glucose-6 phosphate dehydrogenase from a microorganism. This enzyme is sold as a lyophilized white powder.

EC 1.1.1.49

Storage and Stability: Product can be stored at 2~8°C for transportation process up to ten days but long-term storage should be at -20°C.

Alternative Names	: G6PD, Glucose-6-phosphate dehydrogenase (NAD (P)(+))
Unit Definition	: One unit causes the oxidation of one micromole of NADH per minute at 30°C, pH 8.0
Appearance	: White amorphous powder, lyophilized
Molecular Weight	: 55.7 kDa
Activity	: ≥200U / ≥1000U
Pack Size	: 200U / 1000U
Scientific Background	: Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the oxidation of glucose 6-phosphate to 6-phosphogluconolactone, utilizing either NADP ⁺ or NAD ⁺ . It functions as a housekeeping enzyme that plays a vital role in the prevention of cellular damage from reactive oxygen species by providing substrates to prevent oxidative damage ¹ . G6PD deficiency is the most common human enzyme deficiency, affecting upward of 400 million people worldwide and causes premature breakdown of red blood cells ² . Applications of this enzyme include enzymatic determination of NAD ⁺ (NADP ⁺) and G-6-P, and activities of phosphoglucose isomerase, phosphoglucomutase and hexokinase. The enzyme is also used for enzymatic determination of glucose and creatine phosphokinase activity when coupled with hexokinase.
Reference	1. Richardson SR, O'Malley GF. Glucose 6 Phosphate Dehydrogenase Deficiency. [Updated 2022 Sep 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. https://www.ncbi.nlm.nih.gov/books/NBK470315/ 2. Frank JE. Diagnosis and management of G6PD deficiency. Am Fam Physician. 2005 Oct 1;72(7):1277-82. PMID: 16225031.

Glucose-6-Phosphate Dehydrogenase

Activity Assay Protocol

Reaction Components

- 5 mM Glucose-6-Phosphate solution, Sigma G7250 (prepared fresh)
- 0.1 M Tris-HCl, pH 8.0
- 30 mM NAD⁺ solution, Carbosynth NN11969 (prepared fresh)
- 50 mM Enzyme Dilution Buffer (50 mM Tris-HCl buffer, pH 7.5, added 0.1% BSA)

- Step 1** Reconstitute lyophilized enzyme powder with water to desired concentration.
- Step 2** Dilute the reconstituted enzyme preparation to 0.05-0.2 U/mL with the enzyme diluent (D), immediately before the assay.
- Step 3** Prepare the following reaction mixture in a cuvette (d=1.0 cm) and equilibrate at 30°C for about 5 minutes.

1.2 mL	Glucose-6-Phosphate solution	(A)
1.5 mL	Tris-HCl, pH 8.0	(B)
0.3 mL	NAD ⁺ solution	(C)
- Step 4** Add 0.1 mL of the enzyme solution to the reaction mixture and mix gently.

- Step 5** Record the decrease of optical density at 340 nm for 5 minutes in a spectrophotometer. Keep the reaction at a temperature of 30°C.
- Note: Blank the spectrophotometer with water.
- Step 6** Calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).
- Step 7** Measure the blank rate (ΔOD blank) by the same method as the test except that the enzyme diluent (D) is added instead of the enzyme solution.

Unit definition: One unit causes the formation of one micromole of NADH per minute under the conditions described above.

Calculation: Activity can be calculated by using the following formula:

$$\text{Volume Activity (U/mL)} = \frac{\Delta OD/\text{min} (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times V_t \times df}{(6.22 \times 1.0 \times V_s)} = \Delta OD/\text{min} \times 4.98 \times df$$

$$\text{Weight Activity (U/mg)} = (\text{U/mL}) \times 1/C$$

V_t : Total volume (3.1 mL)

V_s : Sample volume (0.1 mL)

6.22 : Millimolar extinction coefficient of NADH ($\text{cm}^2/\text{micromole}$)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration (mg/mL)

