

Glucose-6-Phosphate Dehydrogenase Activity Assay Kit

Cat. No. Kit-0352 **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Glucose-6-Phosphate Dehydrogenase Assay provides a fluorescence-based method for detecting G6PDH activity in a variety of samples including erythrocyte lysates, tissue homogenates, and cell culture samples. In the assay, G6PDH catalyzes the oxidation of glucose-6-phosphate to 6-phospho-D-gluconate, along with the concomitant reduction of NADP⁺ to NADPH. NADPH reacts with the fluorometric detector to yield a highly fluorescent product which can be analyzed with an excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm.

Description

Glucose-6-phosphate dehydrogenase (G6PDH) is a cytosolic enzyme that catalyzes the first step in the pentose phosphate pathway. This pathway includes converting glucose to ribose-5-phosphate, a precursor to RNA, DNA, ATP, CoA, NAD, and FAD. The pathway also generates NADPH. Glutathione reductase uses NADPH to maintain the level of glutathione within the cell, thus protecting the cell from oxidative damage. NADPH is also involved in fatty acid oxidation, lipid biosynthesis, and is the substrate for NADPH oxidase in activated macrophages and polymorphonuclear leukocytes to produce oxygen radicals which destroy pathogens. Most cells have alternate ways of generating intracellular NADPH such as the de novo pathway from amino acids. Since red blood cells do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defense against oxidative damage is dependent on G6PDH. G6PDH deficiency becomes especially lethal in red blood cells, where any oxidative stress will result in hemolytic anemia. G6PDH deficiency, the most common enzyme deficiency worldwide, causes a spectrum of diseases including neonatal hyperbilirubinemia, acute hemolysis, and chronic hemolysis. It is estimated that about 400 million people are affected by this

 Tel: 1-631-559-9269 1-516-512-3133

 Email: info@creative-biomart.com  Fax: 1-631-938-8127

 45-1 Ramsey Road, Shirley, NY 11967, USA

deficiency. Fortunately, most people will remain clinically asymptomatic throughout their lives. The deficiency is an X-linked hereditary genetic defect caused by mutations in the G6PD gene, showing a typical X-linked distribution pattern with higher incidence in males than in females. G6PDH deficiency is known to provide protection against malaria, particularly the deadliest form of malaria caused by *Plasmodium falciparum*. Areas endemic to malaria usually have more individuals with the deficiency, possibly because of an evolutionary advantage. G6PDH activity has been shown to be upregulated in rat and mouse models of obesity, hyperglycemia, and hyperinsulinemia. G6PDH is an important mediator of insulin resistance and is strongly activated post-translationally in (pre)neoplastic lesions to produce NADPH. The tumor suppressor p53, the most frequently mutated gene in human tumors, inhibits G6PDH by binding to the enzyme and preventing the formation of the active dimer.

Usage

Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use.

Storage

Stability: 6 months; Storage: -20°C; This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Kit Components

G6PDH Assay Buffer (10X): 1 vial/5 ml; G6PDH Substrate: 2 vials; G6PDH Cofactor: 2 vials; G6PDH Enzyme Mixture: 2 vials; G6PDH Fluorometric Detector: 2 vials; G6PDH Positive Control: 2 vials/50 µl; NADPH Standard: 2 vials; 96-Well Solid Plate (black): 1 plate; 96-Well Cover Sheet: 1 cover

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