BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Glucose-6-phosphate Dehydrogenase (G6PD) Assay Kit (Cat #: E-110)

COMPONENTS: G6PD Assay Solution- 10 ml (200 wells), store at -80ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The G6PD assay is based on the reduction of the tetrazolium salt INT in a NADPH-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for sensitive detection of G6PD activity in tissue/cell extracts. Assay solution is stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.2 - 1 mg/ml.

Enzyme assay for clear sample:

1. Thaw G6PD Assay Solution and keep solution on ice. Add 10 µl of each sample to a plain (uncoated) 96-well plate. Add 10 µl 1x Cell Lysis Solution to a designated well serving as the blank well.
2. Reaction is initiated by adding 50 µl G6PD Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 30 min or 60 min (do not use CO_2 incubator).
3. Stop reaction by adding 50 µl 3% Acetic acid (not included in the kit) to each well followed by brief gentle agitation.
4. Measure $O.D._{492\ nm}$ using a plate reader. Subtract blank well reading from sample well reading to obtain **ΔO.D.** for each sample.

Enzyme assay for hemolyzed sample:

1. Add 10 µl of each sample to a plain (uncoated) 96-well plate <u>in duplicate</u>.
2. Add 50 µl dH_2O to one set of sample wells (control well). Add 50 µl G6PD Assay Solution to the other set of sample wells (reaction well). Mix contents by brief agitation. Cover plate and incubate at 37°C for 30 min or 60 min (do not use CO_2 incubator).
3. Stop reaction by adding 50 µl 3% Acetic acid (not included in the kit) to each well followed by brief gentle agitation.
4. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading to obtain **ΔO.D.** for each sample.

Calculation of enzyme activity:

For 30 min incubation, G6PD activity in IU/L = µmol/(L•min) = **ΔO.D.** x 1000 x 110 µl / (30 min x 0.6 cm x 18 x 10 µl) = **ΔO.D. x 33.95** (or **16.98** for 60 min incubation). Enzyme activity can be presented as units/µg proteins. Sample protein concentration may be increased to increase ΔO.D..

Additional information

- A solution of 3% Acetic acid needs to be prepared for reaction termination.
- The assay solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for MSDS information.