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

COMPONENTS: G6PD Assay Solution- 5 ml (100 wells), store at -80ºC (**shield solution from light during assay**)
50x G6PD Substrate: 0.1 ml, store at -20ºC or -80ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

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 measurement 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 ~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 (5 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 supernatant protein concentration. A suggested sample protein concentration range is 0.1 – 1 mg/ml. Keep samples on ice during assay.

Reagent thawing:

Keep thawed 50x G6PD Substrate and G6PD Assay Solution on ice. *Do not over thaw*. Shield G6PD Assay Solution from light. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 50 parts of G6PD Assay Solution, e.g. 10 µl dH_2O + 500 µl G6PD Assay Solution. Keep solution on ice.

Reaction solution is prepared by mixing 1 part of 50x G6PD Substrate and 50 parts of G6PD Assay Solution, e.g. 10 µl 50x G6PD Substrate + 500 µl G6PD Assay Solution. Keep solution on ice and use immediately.

Enzyme assay:

1. Add 5 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control well and reaction well).

2. Add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a humidified 37°C incubator for 20 or 40 min. *Do not use CO_2 incubator*.

3. Measure $O.D._{492\ nm}$ using a plate reader.

4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.

5. If incubation for 20 min, enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 55 µl / (20 min × 0.4 cm × 18 × 5 µl) = **ΔO.D. × 76.39**. If incubation for 40 min, enzyme activity = **ΔO.D. x 38.19**.
Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity.

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Redissolve contents by agitation in warm water upon arrival.