BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Glucose-6-phosphate Assay Kit (Cat #: A-119)

COMPONENTS: G6P Assay Solution: 5 ml, store at -80°C **(shield solution from light during assay)**
20 mM G-6-P: 0.1 ml, store at -80°C

PRODUCT DESCRIPTION: Glucose has only one principle fate upon entering the cell: phosphorylation to glucose-6-phosphate (G6P) by hexokinase or glucokinase. G6P is an intermediate in almost every pathway that uses glucose, including glycolysis, the pentose phosphate pathway and glycogen metabolism. The colorimetric G6P assay is based on the reduction of the tetrazolium salt INT in a NADPH-coupled reaction. The INT reaction product exhibits an absorption maximum at 492 nm. The intensity of the red color formed is increased in response to increased G6P (detection limit ~10 µM). The assay solution is stable for several years if stored and handled properly.

PROTOCOLS

G6P Assay Solution:

Quickly thaw G6P Assay Solution. *Do not over thaw solution.* Keep solution on ice and shielded from light. Gently agitate solution prior to first pipetting. Freeze solution immediately after use.

G6P standard:

Thaw 20 mM G6P solution and keep solution on ice. Dilute the 20 mM G6P standard 100-fold with dH_2O to 200 µM (0.2 mM), e.g., 495 µl dH_2O + 5 µl 20 mM G6P. Perform 1:1 serial dilution to generate 100 µM, 50 µM, and 25 µM G6P standards. Store diluted G6P standards at -20°C.

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by TCA precipitation (https://bmrservice.com/deproteination-by-tca).

ASSAY

1. Add 20 µl of each diluted G6P standard and deproteinized sample to a plain (uncoated) 96-well plate.
2. Assay is initiated by addition of 50 µl G6P Assay Solution to each well. Mix contents by gentle agitation for 10 sec.
3. Cover and incubate plate in a 37°C humidified incubator for 60 min. *Do not use CO_2 incubator.*
4. Eliminate air bubbles in wells if necessary. Measure absorbance (O.D.) at 492 nm using a plate reader.
5. Plot G6P standard concentrations vs. respective $O.D._{492\ nm}$ (see graph below). Generate a trend line equation on chart.
6. Calculate sample G6P concentration using the derived equation (x = G6P concentration in µM; y = O.D.492 nm). A new plot must be generated for each assay.

NOTES:

- G6P Assay Solution contains the organic solvent dimethyl sulfoxide (DMSO) and iodonitrotetrazolium chloride. Please contact us or visit the product webpage for SDS information.
- TCA is corrosive and ether is flammable. Exercise caution when handling the reagents.