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

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

COMPONENTS: G6P Assay Solution- 5 ml, store at -70°C **(avoid light exposure)**
20 mM G6P- 0.2 ml, store at -70°C
PEG Solution- 5 ml, store at 4°C **(viscous; pipette solution with a cut tip)**

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 the pathway for glycogen synthesis. 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 5 - 10 μM G6P). The assay solution is stable for several years if stored and handled properly.

PROTOCOLS

G6P Assay Solution:

Quickly thaw G6P Assay Solution and keep solution on ice shielded from light. Gently agitate solution prior to first pipetting. Freeze solution immediately after use.

Glucose-6-P standard:

Quickly thaw 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 additional 1:1 dilution to generate 100 μM, 50 μM, and 25 μM G6P standards. Store diluted standards at -20°C.

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at http://www.bmrservice.com/SupplementPEG.html. Alternatively, the samples can be deproteinized by TCA precipitation (http://www.bmrservice.com/SupplementTCA.html), which is recommended for analysis of nucleotides.

ASSAY

1. Add 20 μl of each G6P standard and deproteinized sample to a plain (uncoated) 96-well plate. Assay reaction is initiated by addition of 50 μl G6P Assay Solution to each well.
2. Mix contents by gentle but thorough agitation for 10 sec. Cover and incubate plate in a 37°C humidified incubator for 60 min. Note: Do NOT use CO_2 incubator.
3. Stop reaction by adding 50 μl 3% acetic acid (not included in the kit) per well followed by brief gentle agitation. Eliminate air bubbles present in wells prior to measurement. Measure absorbance at 492 nm using a plate reader.
4. Plot G6P standards vs. $O.D._{492\ nm}$. Generate a trend line equation on chart. Calculate sample G6P concentration using the derived equation (x = sample G6P concentration in μM; y = $O.D._{492\ nm}$). A new plot must be generated for each assay.

NOTE:

- G6P Assay Solution contains the organic solvent DMSO and iodonitrotetrazolium violet. Please contact us or visit the product webpage for MSDS information.
- A solution of 3% acetic acid needs to be prepared for reaction termination.
- Use extreme caution when performing TCA extraction. TCA is highly corrosive and ether is highly flammable. Please follow the web protocol carefully.