BIOMEDICAL RESEARCH SERVICE CENTER
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Phosphoglucose Isomerase (PGI) Assay Kit (Cat #: E-140)

COMPONENTS: PGI Assay Solution- 5 ml (100 wells), **store in aliquots at -80°C after first thawing**
10x PGI Substrate- 0.5 ml, store at -80°C
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

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

Preparation of cell/tissue extracts:

1. Wash 10^5 – 10^6 cells with ice-cold phosphate-buffered saline (PBS) and remove PBS completely. Cell pellet should be stored at -80ºC. Tissue sample should be washed with PBS thoroughly to remove blood cells.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximal speed. Recover supernatant for assay. Tissue (~25 mg) is homogenized in 0.5 ml 1x Cell Lysis Solution, and lysate is clarified by centrifugation.
3. Perform protein assay to determine sample protein concentration. Normalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution to ~0.1 mg/ml each. Lysate should be stored at -80ºC. Note: Do not use a buffer containing reducing agents or SDS.

Reagent thawing:

Keep thawed PGI Assay Solution, 10x PGI Substrate, and sample on ice. Gently agitate solution prior to pipetting. It is important to minimize the time the reagents are thawed. Freeze solutions immediately after use.

Preparation working assay solution:

Working assay solution is prepared fresh by mixing 1 part of 10x PGI substrate and 10 parts of PGI Assay Solution, e.g. 50 µl 10x PGI Substrate mixed with 500 µl PGI Assay Solution. Keep freshly prepared working assay solution on ice. Discard unused portion.

Enzyme assay:

1. Add 5 µl of each sample (cell lysate, tissue homogenate, culture medium, or plasma/serum) to a 96-well plate. Note: For drug discovery application, add 1 µl of a drug inhibitor to well and mix with sample by pipetting up and down.
2. Add 50 µl ice-cold working assay solution to an empty well. This is the background well.
3. Swiftly add 50 µl ice-cold working assay solution to each sample well. Gently agitate plate to mix contents for 10 sec.
4. Cover plate and incubate in a humidified 37°C incubator for 10 min or 20 min. Do not use CO_2 incubator. Reaction generates cherry red color in wells.
5. Stop assay by adding 50 µl 3% Acetic acid (not included in the kit) to the background well and each sample well followed by brief gentle agitation.
6. Measure $O.D._{492\ nm}$ using a plate reader. Subtract background well reading from each sample well reading. Use the subtracted sample reading (**O.D.**) for enzyme activity calculation shown below.
   Note: As a rule of thumb, samples should be diluted with ice-cold 1x Cell Lysis Solution if the O.D. of sample wells exceeds 1.5. Alternatively, reaction time can be shortened.
7. If assay for 10 min, sample PGI activity in IU/L unit = µmol/(L•min) = O.D. × 1000 × 105 µl / (10 min × 0.6 cm × 18 × 5 µl) = **O.D. × 194.4.** If assay for 20 min, PGI activity = **O.D. x 97.2**. Multiply result by sample dilution factor where applicable.

Additional information:

- The assay is sensitive to color arising from sample hemolysis. A sample control well (containing 5 µl sample and 50 µl dH_2O) can be included for O.D. correction. The sample control O.D. should also be subtracted from the sample reading.
- A 3% Acetic acid solution 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.