BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO
Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Phosphoglucose Isomerase (PGI, GPI, AMF) Assay Kit (Cat #: E-140)

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

PRODUCT DESCRIPTION: The phosphoglucose isomerase (PGI, GPI, or AMF) activity assay kit is based on NADPH-dependent reduction of INT to formazan, which exhibits an absorption maximum at 492 nm (extinction coefficient = 18 $mM^{-1}cm^{-1}$), allowing for sensitive measurement of PGI activity present in cells/tissues, culture medium, and biological fluids such as serum/plasma, saliva, and urine. Kit components are 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 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 (~2 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 lysate protein concentration range is 0.05 – 0.2 mg/ml. *Normalization of lysate protein concentration is recommended.*

Preparation of reaction and control solutions:

Keep thawed PGI Assay Solution on ice and shielded from light. *Do not over thaw.*

Control solution is prepared by mixing 1 part of dH_2O and 50 parts of PGI Assay Solution, e.g., 10 µl dH_2O and 500 µl PGI Assay Solution.

Reaction solution is prepared by mixing 1 part of 50x PGI substrate and 50 parts of PGI Assay Solution, e.g., 10 µl 50x PGI Substrate and 500 µl PGI Assay Solution. Keep both reaction and control solutions on ice.

Dilution of serum/plasma samples:

Dilute serum/plasma 5-fold with dH_2O, e.g., 40 µl dH_2O and 10 µl serum/plasma. Mix well and place on ice.

Enzyme assay:

1. Add 5 µl of each sample to duplicate wells of a 96-well plate (for control and reaction).
2. Add 50 µl control solution to each well of the control set and 50 µl reaction solution to each well of the reaction set. Gently agitate plate for 10 sec.
3. Cover and incubate plate in a 37°C incubator for 20 min. *Do not use CO_2 incubator.*
4. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample to obtain **ΔO.D.**.
5. PGI activity in IU/L = µmol/(L•min) = ΔO.D. × 1000 × 55 µl / (20 min × 0.3 cm × 18 × 5 µl) = **ΔO.D. × 101.85**
Cell/tissue PGI activity may be normalized protein concentration or by GAPDH enzyme activity (see #E-101).

Additional information:

- PGI 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. Re-dissolve contents by agitation in warm water upon arrival.