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

Cytosolic Glycerol-3-Phosphate Dehydrogenase (cGPDH) Assay Kit (Cat#: E-122c)
Mitochondrial Glycerol-3-Phosphate Dehydrogenase (mGPDH) Assay Kit (Cat#: E-122m)

COMPONENTS: cGPDH or mGPDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light)**
50x GPDH Substrate- 0.1 ml, store at -20ºC or -80ºC
10x Lysis Buffer: 25 ml, store at 4°C
10x Extraction Buffer: 20 ml, store at 4°C

PRODUCT DESCRIPTION: The cGPDH or mGPDH enzyme activity assay is based on reduction of iodonitrotetrazolium in a NADH- or $FADH_2$-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$), allowing for measurement of cGPDH or mGPDH activity in cell/tissue lysates and serum/plasma. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue lysates:

1. **Cells**: Bring up ~10^5 freeze-thawed cells in ~0.1 ml ice-cold 1x Lysis Buffer by pipetting up and down. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet in ~50 µl ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the pellet is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization. Centrifuge homogenate at ~5000 rpm for 1 min. Harvest the supernatant for GPDH assay. Recommended sample protein concentration is 0.2 – 2 mg/ml. *Normalization of sample protein concentration is recommended.*
2. **Tissue:** Mechanically homogenize freeze-thawed tissue in ice-cold 1x Lysis Buffer (~5 mg tissue in 0.5 ml). Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet in ~0.25 ml ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the pellet is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization. Centrifuge homogenate at ~5000 rpm for 1 min. Harvest the supernatant for GPDH assay. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Keep thawed assay solution and substrate on ice and shielded from light. *Do not over thaw.* 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 GPDH Assay Solution, e.g. 10 µl dH_2O + 500 µl Assay Solution.

Reaction solution is prepared by mixing 1 part of 50x GPDH Substrate and 50 parts of GPDH Assay Solution, e.g. 10 µl 50x GPDH Substrate + 500 µl Assay Solution.

Enzyme assay:

1. Thaw samples quickly and keep on ice. *Do not over thaw.* Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate. *For serum/plasma samples, add 10 µl to duplicate wells.*
2. Add 50 µl control solution to one set of sample wells (control set) and 50 µl reaction solution to the other set of sample wells (reaction set). Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 min or 90 min. *Do not use CO_2 incubator.*
3. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
4. If incubation for 60 min, GPDH enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl/(60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1**. If incubation for 90 min, GPDH activity = **ΔO.D. x 5.4**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

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.