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Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Assay Kit (Cat #: E-101)

COMPONENTS: GAPDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
50x GAPDH Substrate- 0.1 ml, store at -20ºCor -80ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The GAPDH enzyme activity assay is based on the reduction of INT in a NADH-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for measurement of GAPDH activity in tissue/cell extracts. 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 (~5 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 sample protein concentration range is 0.1 – 2 mg/ml. *Normalization of lysate protein concentration is recommended.*

Reagent thawing:

Keep thawed GAPDH Assay Solution and 50x GAPDH Substrate on ice. *Do not over thaw*. Shield GAPDH Assay Solution from light. 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 GAPDH Assay Solution, e.g. 10 µl dH_2O mixed with 500 µl GAPDH Assay Solution.

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

Enzyme assay:

1. Add 10 µl of each sample to duplicate wells of a plain (uncoated) 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. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 30 min or 60 min. *Do not use CO_2 incubator*. Cherry red color should gradually appear in wells.
3. Measure $O.D._{492\ nm}$ using a plate reader.
4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
5. If incubation for 30 min, GAPDH enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 60 µl / (30 min × 0.4 cm × 18 × 10 µl) = **ΔO.D. × 27.78**. If incubation for 60 min, GAPDH activity = **ΔO.D. x 13.89**. Enzyme activity may be normalized by protein concentrations. Increase sample protein concentration if detected enzyme activity is too low.

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.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.