BIOMEDICAL RESEARCH SERVICE CENTER
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Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Assay Kit (Cat #: E-101)

COMPONENTS: GAPDH Assay Solution- 5 ml (for 100 wells); **store in aliquots at -80°C after first thawing**
10x Cell Lysis Solution- 25 ml, store at 4℃

PRODUCT DESCRIPTION: The GAPDH assay is based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to INT-formazan, which exhibits an absorption maximum at 492 nm (molar extinction coefficient = 18 $mM^{-1}cm^{-1}$) and allows for sensitive detection of GAPDH activity in crude serum and tissue samples. Assay solution is stable for several years if stored and handled properly.

Plasma: Plasma samples may need to be diluted with ice-cold 1x Cell Lysis Solution (from 10-fold dilution of 10x Cell Lysis Solution with dH_2O) to obtain assay linearity. Samples should be store at -80℃.

Preparation of cell/tissue extracts:

1. Wash $10^5 - 10^6$ cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80℃ until use.
2. 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 maximum speed (~13,000 rpm) and harvest supernatant for GAPDH assay. For tissue extraction, weigh ~50 mg tissue and homogenize in 1 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4℃ for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80℃.
3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.2 – 2 mg/ml.

*** Single time point assay:**

1. Thaw GAPDH Assay Solution and keep on ice. Agitate solution prior to pipetting. Add 10 µl sample to a 96-well plate. Use 10 µl 1x Cell Lysis Solution as blank. Reaction is initiated by adding 50 µl GAPDH Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 30 min or 60 min (for more intense reaction).
2. Stop reaction by adding 50 µl 3% Acetic acid (not included in the kit) to each well followed by brief gentle agitation. Measure $O.D._{492\ nm}$ using a plate reader. Subtract blank reading from sample reading. If incubation for 30 min, GAPDH activity in IU/L unit = µmol/(L•min) = O.D. x 1000 x 110 µl / (30 min x 0.6 cm x 18 x 10 µl) = **O.D. x 33.95**. If incubation for 60 min, GAPDH activity in IU/L unit = **O.D. x 16.98**

**** Dual time point assay:** Use this format to eliminate interference that may be caused by sample hemolysis.

1. Set up assay in duplicate 96-well plates for two selected time points. We suggest using 20 and 40 min initially. Longer assay time can be adopted if weak GAPDH activity is observed (e.g. 30 & 50 min or 40 & 60 min). For samples exhibiting high GAPDH activity, select two time points with a 10-min interval (e.g. 10 & 20 min or 20 & 30 min). Optimal assay time may be determined empirically.
2. For the 20/40 min format, add 10 µl sample to each of the two 96-well plates. Include a 10 µl blank on each plate. Add 50 µl GAPDH Assay Solution to each well and mix by brief gentle agitation. Cover plates and incubate at 37°C for 20 min. Remove one plate and stop reaction by adding 50 µl 3% Acetic acid. Remove the second plate at 40 min and stop reaction by Acetic acid. Measure $O.D._{492\ nm}$. Subtract blank reading from sample reading. GAPDH activity in IU/L unit = $(O.D._{40\ min} - O.D._{20\ min})$ x 1000 x 110 / (20 x 0.6 x 18 x 10) = **ΔO.D. x 50.93**. If using a 10-min interval, GAPDH activity in IU/L unit = **ΔO.D. x 101.85**

Additional information:

- Interference caused by sample hemolysis can also be eliminated by including a sample control well containing 10 µl sample, 50 µl dH_2O and 50 µl 3% Acetic acid. The control well reading is then subtracted from sample reaction reading.
- Note that "0.6 cm" in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- 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.