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Inosine-5′-monophosphate Dehydrogenase (IMPDH) Assay Kit (Cat #: E-119)

COMPONENTS: IMPDH Assay Solution- 5 ml (for 100 wells), store at -80°C **(shield solution from light during assay)**
50x IMPDH Substrate- 0.1 ml, store at -80°C
10x Sample Buffer- 25 ml, store at 4°C

PRODUCT DESCRIPTION: The IMPDH enzyme activity assay is based on 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 IMPDH activity in cell/tissue lysates. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. PBS-washed cell/tissue samples should be frozen at -80°C prior to homogenization. At least ~10^5 cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction.
2. Prepare 1x Sample Buffer by diluting 10x Sample Buffer with dH_2O. Bring up freeze-thawed cells in 50–100 µl ice-cold 1x Sample Buffer by pipetting up and down. Adherent cells after deep freezing can be directly harvested from plate by scraping. Leave lysate on ice for 5 min with agitation. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Sample Buffer (~5 mg tissue in 500 µl).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
4. Perform protein assay to determine lysate (supernatant) protein concentration. A suggested sample protein concentration range is 1 – 3 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Keep thawed IMPDH Assay Solution and 50x IMPDH Substrate on ice. *Do not over thaw.* Keep IMPDH Assay Solution shielded 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 IMPDH Assay Solution, e.g. 10 µl dH_2O + 500 µl IMPDH Assay Solution.

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

Enzyme assay:

1. Thaw lysates 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 control and reaction wells.
2. Add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a humidified 37°C incubator for 60 min or 90 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. 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, IMPDH 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, IMPDH 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.