BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

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

COMPONENTS: IMPDH Assay Solution- 5 ml (100 assays), **store in aliquots at -80°C after first thawing**
50x IMPDH Substrate- 0.1 ml, store at -80°C
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

PRODUCT DESCRIPTION: The IMPDH enzyme activity assay is based on the reduction of INT in a NADH-coupled reaction to INT-formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for sensitive measurement of IMPDH activity both in vivo and in vitro. Assay solution is stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Wash ~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 -80ºC until use.
2. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution (diluted 10-fold with dH_2O from 10x 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 IMPDH assay. For tissue extraction, weigh ~50 mg tissue and homogenize in 1 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4ºC for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80ºC.
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 1 – 2 mg/ml. Since the assay is sensitive to protein concentration, a pilot study using samples of varying protein concentrations should be carried out to optimize the assay condition.

Reagent thawing:

Keep thawed IMPDH Assay Solution and 50x IMPDH Substrate on ice. Gently agitate solution prior to pipetting. It is important to minimize the time the reagents are thawed. 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 mixed with 500 µl IMPDH Assay Solution. Keep freshly prepared control solution on ice during assay.

Reaction solution is prepared by mixing 1 part of IMPDH substrate and 50 parts of IMPDH Assay Solution, e.g. 10 µl 50x IMPDH Substrate mixed with 500 µl IMPDH Assay Solution. Keep freshly prepared reaction solution on ice during assay.

Each enzyme sample will be treated with 50 µl control solution and 50 µl reaction solution in two separate sets. Estimate the volume of control solution and reaction solution required for each assay.

Enzyme assay:

1. Add each enzyme sample (15 µl per well) to a 96-well plate in duplicate. Note: For drug discovery application, add 1 µl of inhibitor to both wells. Mix enzyme and inhibitor by pipetting up and down.
2. After all samples have been pipetted to the plate, swiftly add 50 µl control solution to one set of wells and 50 µl reaction solution to another set of wells. Mix contents by gentle agitation for 30 sec. Cover plate and incubate in a humidified 37°C incubator for 1 hour or 2 hours. Do not use CO_2 incubator.
3. Stop assay by adding 50 µl 3% Acetic acid (not included in the kit) to each control solution well and reaction solution well followed by brief gentle agitation. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted sample reading (**O.D.**) for enzyme activity calculation shown in step 4.
4. If assay for 1 hour, sample IMPDH activity in IU/L unit = µmol/(L•min) = O.D. × 1000 × 115 µl / (60 min × 0.6 cm × 18 × 15 µl) = **O.D. × 11.83 (**or **x 5.92** for 2-hour assay**)**. Multiply result by the enzyme dilution factor where applicable. Sample protein concentration should be increased if low enzyme activity is observed.

Additional information:

- The "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.