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GMP Synthase (GMPS) Assay Kit (E-160)

COMPONENTS: GMPS Buffer- 5 ml, store at -80ºC
GMPS Substrate- 1 ml, store at -80ºC
TA Assay Solution- 5 ml (for 100 wells); store at -80ºC (**shield solution from light during assay**)
Glutamine- 50 mg, store at 4ºC
10x Sample Buffer- 25 ml, store at 4ºC

PRODUCT DESCRIPTION: The GMP synthase (GMPS) enzyme activity assay is based on sequential GMPS- and glutamate dehydrogenase-mediated reactions, the latter of which is coupled to reduction of INT to INT-formazan (molar extinction coefficient ε= 18 $mM^{-1}cm^{-1}$ at 492 nm), allowing for measurement of GMPS activity present in crude cell/tissue lysates. Kit components are stable for several years if handled and stored properly.

Preparation of cell/tissue extracts:

1. PBS-washed cell/tissue samples should be frozen at -80ºC 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 0.2 – 2 mg/ml. *Normalization of sample protein concentration is recommended.*

Preparation of glutamine solution: Assay of each sample requires 40 µl glutamine solution. Prepare a fresh 5 mg/ml glutamine solution in GMPS Buffer, e.g., 2.5 mg in 0.5 ml GMPS Buffer. Vigorously vortex solution to dissolve glutamine. Discard unused glutamine solution.

Preparation of control solution and reaction solution: Assay of each sample requires 40 µl control solution and 40 µl reaction solution. Prepare control solution by mixing an equal volume of fresh glutamine solution and dH_2O. Prepare reaction solution by mixing an equal volume of fresh glutamine solution and GMPS Substrate.

TA Assay Solution: Keep thawed GMPS Substrate and TA Assay Solution on ice. Shield TA Assay Solution from light. *Do not over thaw.* Freeze both solutions immediately after use.

Enzyme assay:

1. Use a plain (uncoated) 96-well plate for the assay. Pipette 20 µl of each sample to duplicate wells serving as control well and reaction well.
2. Add 40 µl control solution to each control well. Add 40 µl reaction solution to each reaction well. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a humidified 37ºC incubator for 60 min. *Do not use CO_2 incubator.*
3. Remove plate from incubator. Add 50 µl TA Assay Solution to each control well and each reaction well. Gently agitate plate to mix. Cover plate and incubate in a humidified 37ºC incubator for another 60 min.
4. Remove plate from incubator. Measure O.D.492 nm using a plate reader.
5. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation as shown below.
6. GMPS enzyme activity in IU/L = µmol/(L•min) = (**ΔO.D.** × 1000 × 110 µl) ÷ (60 min × 0.5 cm × 18 × 20 µl) = **ΔO.D.× 10.19**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (Cat#E-101).

Additional information:

The kit contains DMSO, INT, and Tris. Please refer to the product webpage or contact us for SDS information.