BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Glutamine F-6-P amidotransferase (GFAT) Assay Kit (Cat #: E-104)

COMPONENTS: GFAT Buffer- 5 ml, store at -80ºC
GFAT 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 room temperature
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The glutamine fructose-6-phosphate amidotransferase (GFAT) enzyme activity assay is based on sequential GFAT- and glutamate dehydrogenase-mediated reactions, the latter of which is coupled to reduction of iodonitrotetrazolium to formazan (molar extinction coefficient $\varepsilon = 18\ mM^{-1}cm^{-1}$ at 492 nm), allowing for measurement of GFAT 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. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold 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. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (use ~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 0.2 – 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 GFAT Buffer, e.g., 2.5 mg glutamine in 0.5 ml GFAT 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 GFAT Substrate.

TA Assay Solution: Keep thawed TA Assay Solution on ice and shielded from light. *Do not over thaw.* Freeze solution 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 one set of sample wells (control). Add 40 µl reaction solution to the other set of sample wells (reaction). 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. GFAT enzyme activity in IU/L = µmol/(L•min) = (**ΔO.D.** × 1000 × 110 µl) ÷ (60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D.× 12.73**.
   Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (Cat#E-101).

Additional information:

- The kit contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.