BIOMEDICAL RESEARCH SERVICE CENTER
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Glutaminase (GLS) Assay Kit (Cat #: E-133)

COMPONENTS: Glutaminase Buffer- 10 ml, store at 4ºC
Glutamine- 0.3 g, store at room temperature
TA Assay Solution- 10 ml (for 200 wells); store at -70ºC **(shield solution from light during assay)**
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: The GLS assay is based on sequential Glutaminase-mediated hydrolytic reaction and glutamate dehydrogenase reaction, which couples the reduction of INT to INT-formazan (ε = 18 $mM^{-1}cm^{-1}$ at 492 nm), allowing for detection of GLS enzyme activity in crude samples. The assay should be optimized for each sample type. 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 at least ~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. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 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 1 – 2 mg/ml.

Preparation of Glutamine solution: Prepare fresh a 15 mg/ml Glutamine solution in Glutaminase Buffer for each assay. Vigorously vortex solution to completely dissolve glutamine. Keep solution at room temperature. Discard solution after each assay.

TA Assay Solution: Keep thawed TA Assay Solution on ice. Minimize the time that the solution is thawed. Freeze TA Assay Solution immediately after use.

Enzyme activity assay:

1. Each sample to be assayed has a control well (containing 10 µl sample and 40 µl dH_2O) and a reaction well (containing 10 µl sample and 40 µl freshly prepared Glutamine solution). Add sample to a plain (uncoated) 96-well plate in duplicate: one set for control wells and another set for reaction wells.
2. After all samples have been pipetted to the plate in duplicate, add 40 µl dH_2O to each well of the control set and 40 µl Glutamine solution to each well of the reaction set. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a humidified 37°C incubator for 2 hours (do not use CO_2 incubator). This incubation step allows the hydrolytic reaction to take place.
3. Remove plate from the incubator. Add 50 µl TA Assay Solution to each control and reaction well. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a humidified 37°C incubator for 1 hour. This step measures the amount of glutamate released by glutaminase.
4. Remove plate from the incubator. Terminate reaction by adding 50 µl 3% Acetic acid (not included in the kit) to each control and reaction 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 reading (**ΔO.D.**) for enzyme activity calculation as shown below.
5. GLS activity in IU/L = µmol/(L•min) = (**ΔO.D.** × 1000 × 150 µl) ÷ (120 min × 0.7 cm × 18 × 10 µl) = **ΔO.D. × 9.92**. Sample enzyme activity can be presented as units/µg proteins.

Additional information:

- A solution of 3% acetic acid 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.