BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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GABA Aminotransferase (GABAT) Assay Kit (Cat #: E-134)

COMPONENTS: GABAT Substrate- 2 ml, store at -80ºC
TA Assay Solution- 5 ml (100 wells); store -80ºC **(shield solution from light during assay)**
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The GABAT activity assay is based on sequential GABAT-mediated transamination reaction and glutamate dehydrogenase reaction, which couples the reduction of iodonitrotetrazolium to formazan (extinction coefficient = 18 $mM^{-1}cm^{-1}$ at 492 nm), allowing for measurement of GABAT enzyme activity in cell/tissue extracts. Kit components are stable for several years if stored and handled 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 0.5 – 4 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent handling:

Keep thawed protein samples, GABAT Substrate, and TA Assay Solution on ice. *Do not over thaw*. Keep TA Assay Solution shielded from light. Freeze all solutions immediately after use.

Assay Protocol:

1. Each sample to be assayed has a control well (containing 20 µl sample and 40 µl dH_2O) and a reaction well (containing 20 µl sample and 40 µl GABAT Substrate). First pipette 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate.

2. Add 40 µl dH_2O to each well of the control set and 40 µl GABAT Substrate to each well of the reaction set. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a 37°C incubator for 60 min. *Do not use CO_2 incubator.*

3. Remove plate from the incubator. Add 50 µl TA Assay Solution to each control well and reaction well. Gently agitate plate to mix. Cover plate and incubate in a 37°C incubator for another 60 min.

4. 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. GABAT enzyme activity in IU/L = µmol/(L•min) = (**ΔO.D.**. × 1000 × 110 µl) ÷ (60 min × 0.5 cm × 18 × 20 µl) **= ΔO.D. × 10.185**. 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.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.