

BIOMEDICAL RESEARCH SERVICE CENTER

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

COMPONENTS: GABAT Substrate- 5 ml, store at -80°C
TA Assay Solution- 10 ml (for 200 wells); store at -80°C (**shield solution from light during assay**)
10x Cell Lysis Solution- 25 ml, store at 4°C (**contains 1% TX-100; swirl bottle briefly prior to dilution**)

PRODUCT DESCRIPTION: The GABAT assay is based on sequential GABAT-mediated transamination reaction and glutamate dehydrogenase reaction, which couples the reduction of INT to formazan (extinction coefficient = $18 \text{ mM}^{-1}\text{cm}^{-1}$ at 492 nm), allowing for sensitive detection of GABAT enzyme activity in cell/tissue extracts. Assay solutions 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 dH₂O. Bring up at least $\sim 10^5$ 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 ($\sim 10 \text{ mg}$ tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at $\sim 14,000 \text{ rpm}$ for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 – 2 mg/ml.

Reagent handling:

Keep thawed GABAT Substrate and TA Assay Solution on ice. It is important to minimize the time that the solution is thawed. Freeze solution immediately after use.

Assay Protocol:

1. Each sample to be assayed has a control well (containing 10 μl sample and 40 μl dH₂O) and a reaction well (containing 10 μl sample and 40 μl GABAT Substrate). Pipette 10 μl of each sample to a plain (uncoated) 96-well plate in duplicate: one control set and one reaction set.
2. After all samples have been pipetted to the plate in duplicate, add 40 μl dH₂O 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 CO₂ incubator). This incubation step allows the transamination reaction to take place.
3. Remove plate from the incubator. Add 50 μl TA Assay Solution to each control well and reaction well. Gently agitate plate for 10 sec to mix. Cover plate and incubate in a 37°C incubator for another 60 min. This step measures the amount of glutamate generated by GABAT.
4. Remove plate from the incubator. Terminate reaction by adding 50 μl 3% Acetic acid (not included in the kit) to each control well 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 ($\Delta\text{O.D.}$) for enzyme activity calculation as shown below.
5. GABAT enzyme activity in IU/L = $\mu\text{mol}/(\text{L}\cdot\text{min}) = (\Delta\text{O.D.} \times 1000 \times 150 \mu\text{l}) \div (60 \text{ min} \times 0.7 \text{ cm} \times 18 \times 10 \mu\text{l}) = \Delta\text{O.D.} \times 19.84$. 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.