BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Aspartate Aminotransferase (AST/GOT) Assay Kit (Cat #: E-116)

COMPONENTS: AST Substrate- 2 ml, store at -80ºC
TA Assay Solution- 5 ml (100 wells), store at -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 enzyme activity assay is based on sequential AST-mediated transamination reaction and glutamate dehydrogenase reaction, which couples the reduction of INT to formazan for measurement of AST activity in cell/tissue lysates. Kit components are stable for at least one year if stored and handled properly.

Sample preparation:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with 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 1 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. Dilute lysate protein concentration to 0.1 - 0.5 mg/ml using ice-cold 1x Cell Lysis Solution. Normalization of lysate protein concentration is recommended.

4. Serum/plasma sample should be diluted 10-fold with ice-cold 1x Cell Lysis Solution prior to assay, e.g., 90 µl 1x Cell Lysis Solution + 10 µl sample. Do not use undiluted serum/plasma for the assay.

Reagent handling:

Keep thawed protein samples, AST Substrate and TA Assay Solution on ice. *Do not over thaw solutions.* TA Assay Solution should be shielded from light. Freeze 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 AST Substrate). Pipette 10 µl of each 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 control well and 40 µl AST Substrate to each reaction well. 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 and reaction well. Gently agitate plate 10 sec to mix. Cover plate and incubate in a 37°C incubator for another 60 min.

4. Remove plate from incubator and allow plate to cool down for ~5 min. 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. AST enzyme activity in IU/L unit = µmol/(L•min) = (**ΔO.D.** × 1000 × 100 µl) ÷ (60 min × 0.5 cm × 18 × 10 µl) **= ΔO.D. × 18.52**. 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 SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.