# *BIOMEDICAL RESEARCH SERVICE CENTER*
# *UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK*

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

**COMPONENTS**: AST Substrate- 5 ml, store at -70ºC
TA Assay Solution- 10 ml (for 200 wells), store at -70ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

**PRODUCT DESCRIPTION:** The assay is based on sequential AST-mediated transamination reaction and glutamate dehydrogenase reaction, which couples the reduction of INT to formazan ($\varepsilon$ = 18 $mM^{-1}cm^{-1}$) for detection of AST activity in cell/tissue extracts and serum/plasma. 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 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. Dilute sample protein concentration to 0.1 mg/ml using ice-cold 1x Cell Lysis Solution.

**Dilution of serum/plasma:**

Each 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). Mix well. Use 5 µl diluted sample for AST assay. Do not use undiluted serum/plasma for the assay.

**Reagent thawing:**

Keep thawed AST Substrate and TA Assay Solution on ice (do not over thaw). Vortex substrate solution to re-dissolve contents if necessary. Minimize the time that the reagents are thawed. Freeze solutions immediately after use.

**Enzyme activity assay:**

1. Each sample to be assayed has a control well (containing 5 µl sample and 45 µl $dH_2O$) and a reaction well (containing 5 µl sample and 45 µl AST Substrate). Pipette 5 µl of each sample to a 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 45 µl $dH_2O$ to each control well and 45 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. This incubation step allows transamination 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 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 AST.
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 sample reading (**ΔO.D.**) for enzyme activity calculation as shown below.
5. AST enzyme activity in IU/L unit = µmol/(L·min) = (**ΔO.D.** × 1000 × 150 µl) ÷ (60 min × 0.7 cm × 18 × 5 µl) = **ΔO.D. × 39.68**. For tissue/cell extracts, 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.