BIOMEDICAL RESEARCH SERVICE CENTER
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Aspartate Aminotransferase (AST/GOT) Assay Kit (Cat #: E-116)

COMPONENTS: AST Substrate- 5 ml, store at -80℃ 10x Cell Lysis Solution- 25 ml, store at 4℃
AST Assay Solution- 10 ml (for 200 wells); **store in aliquots at -80°C after first thawing**

PRODUCT DESCRIPTION: The assay is based on sequential AST-mediated transamination 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 sensitive detection of AST enzyme activity in plasma and cell/tissue samples. Assay solution is stable for several years if stored and handled properly.

Cell Lysis Solution: Dilute an aliquot of 10x Cell Lysis Solution 10-fold with ice-cold dH_2O to obtain 1x Cell Lysis Solution (e.g. 0.9 ml dH_2O + 0.1 ml 10x Cell Lysis Solution). Keep solution on ice.

Preparation of plasma samples: Each 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 plasma). Mix well; use 5 µl (or 10 µl) diluted plasma for AST assay. Samples should be stored at -80℃. DO NOT use undiluted plasma for the assay.

Preparation of cell/tissue extracts:

1. Wash $10^5 - 10^6$ cells with ice-cold phosphate-buffered saline (PBS; not provided). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80℃ until use.
2. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm). Harvest supernatant for AST assay. For tissue extraction, homogenize ~50 mg of tissue in 1 ml 1x Cell Lysis Solution. Centrifuge homogenate at 4℃ for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80℃.
3. Perform protein assay to determine sample protein concentration. Dilute sample with 1x Cell Lysis Solution to 0.1 mg/ml. Use 5 µl (or 10 µl) of each protein sample for AST assay.

Reagent thawing:

Keep thawed AST Substrate and AST Assay Solution on ice. Gently agitate solution prior to pipetting. It is important to 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 sample and 45 µl dH_2O) and a reaction well (containing sample and 45 µl AST Substrate). Pipette 5 µl (or 10 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 30 sec to mix. Cover plate and incubate in a humidified 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 AST Assay Solution to each control and reaction well. Gently agitate plate for 30 sec to mix. Cover plate and incubate in a humidified 37°C incubator for another 60 min. This step measures the amount of glutamate generated by AST at step 2.
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 (**O.D.**) for enzyme activity calculation as shown below.
5. If using 5 µl sample for assay, AST activity in IU/L unit = µmol/(L•min) = (O.D. × 1000 × 150 µl) ÷ (60 min × 0.6 cm × 18 × 5 µl) **= O.D. × 46.30**. If using 10 µl sample for assay, AST activity = (O.D. × 1000 × 155 µl) ÷ (60 min × 0.6 cm × 18 × 10 µl) = **O.D. × 23.92**.

Additional information:

- A 3% Acetic acid solution needs to be prepared for reaction termination.
- Note that "0.6 cm" in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- The assay solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for MSDS information.