AST / Aspartate Transaminase Assay Kit

AST / Aspartate Transaminase Assay Kit is an assay kit for the quantification of AST / Aspartate Transaminase in serum and plasma.

Catalog number: ARG81297

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Aspartate transaminase (AST) or aspartate aminotransferase, also known as AspAT/ASAT/AAT or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme that was first described by Arthur Karmen and colleagues in 1954. AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Serum AST level, serum ALT (alanine transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health. The tests are part of blood panels.

AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma [Wikipedia]

PRINCIPLE OF THE ASSAY

AST is an aminotransferase that catalyzes the reversible transfer of the amino group from glutamate to oxaloacetate. And then oxaloacetate and NADH are converted to malate and NAD by malate dehydrogenase and this step is irreversible reaction. The assay kit is an indirect enzymatic method to detect the decrease level of NADH by absorbance at 340 nm and it is proportionate to AST activity. The total incubation time is 10 min only.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	24 ml	-20°C
Enzyme Mixture	120 µl	-20°C
Cofactor	120 µl	-20°C
NADH	1 vial (lyophilized)	-20°C

The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt, 3 weeks after reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 340 nm
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Heat block or hot water bath or oven
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at r-20°C at all times.
- Keep thawed Enzyme Mixture on ice before use.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles.

Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- **NADH Reagent:** Reconstitute NADH with **1000 µl** of **distilled water**, to yield a concentration of **10 mM**. Unused reconstituted NADH reagent is stable for three weeks when stored at -20°C.
- **Sample / Standard Working Reagent:** *Prepare before use,* for each well mix the following reagents:

  200 µl Assay Buffer,

  1 µl Cofactor,

  1 µl Enzyme Mixture

  4 µl of reconstituted NADH

  Warm to assay temperature (e.g. RT or 37°C) before use. Add 200 µl per well in sample and standard wells.
- **Blank Working Reagent:** *Prepare before use,* for each well mix the following reagents:

  200 µl Assay Buffer,

  1 µl Cofactor,

  1 µl Enzyme Mixture

  4 µl of distilled water

  Warm to assay temperature (e.g. RT or 37°C) before use. Add 200 µl per well in blank wells.
- **Enzyme Mixture:** Keep thawed enzyme on ice and return unused Enzyme to ice or -20°C immediately.
- **Assay buffer:** Assay buffer is ready to use, mix it well by vigorous shaking before use.

ASSAY PROCEDURE

Working reagents, microplate and spectrophotometer should be equilibrated to assay temperature before use, the kit could be assayed at room temperature (RT) or 37°C. Standards and samples should be assayed in at least duplicates.

1. Add **20 µl** of **distilled water** in standard and blank wells.
2. Add **20 µl** of **samples** into the corresponding sample wells. Keep plate at the assay temperature.
3. Prepare Sample / Standard Working Reagent for Sample and Standard and Blank Working Reagent for blank.
4. Add **200 µl** of **Sample / Standard Working Reagent** in the Sample and Standard wells.
5. Add **200 µl** of **Blank Working Reagent** in the Blank wells.
6. Tap the plate to mix it well immediately. **Incubation the plate in the assay temperature (RT or 37°C)**
7. **Read O.D.** with a microplate reader **at 340 nm** at **5 min** and **10 min** after incubate in the assay temperature.

Summary of AST / Aspartate Transaminase Procedure

Reagent	Sample	Standard	Blank
Distilled water	-	20 µl	20 µl
Sample	20 µl	-	-
Keep plate at the assay temperature. (e.g. RT or 37°C).			
Sample/Standard Working Reagent	200 µl	200 µl	-
Blank Working Reagent	-	-	200 µl
Tap the plate to mix it well immediately.			
Incubation the plate in the assay temperature (RT or 37°C)			
Read O.D. with a microplate reader at 340 nm at 5 min and at 10 min.			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.

2. For each Sample and standard, calculate the rate of NADH consumption by subtracting the OD at 10 min from the OD at 5 min. The descriptions are as below.

ΔOD_{Sample} = (OD_{Sample} at 5 min)- (OD_{Sample} at 10 min)

ΔOD_{NADH} = ($OD_{Standard}$ at 5 min)- ($OD_{Standard}$ at 10 min)

$OD_{Standard5}$ = $OD_{Standard}$ at 5 min

OD_{Blank} = OD_{Blank} at 5 min

3. Determine AST activity using the following equation:

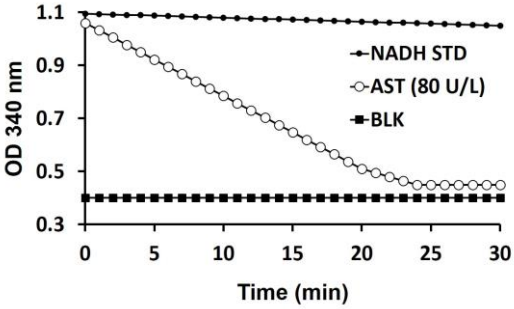
$$AST\ (U/L) = 388 \times (\Delta OD_{Sample} - \Delta OD_{NADH}) / (OD_{Standard5} - OD_{Blank})$$

Note: the factor 388 comes from:
10 mM NADH X [4 µl (Vol.NADH)/206 µl (Vol.WorkReagent)]
X [200 µl (Vol.WorkReagent)/220 µl (Vol.Total)] X [11 (sample dilution)/5 min]
= 0.388 mM/min = 338 µM/min

4. If the calculated AST activity is higher than 100 U/L, dilute sample in Assay Buffer and repeat assay. Multiple results by the dilution factor.

5. Unit definition: 1 Unit (U) of AST will catalyze the conversion of 1 µmole of aspartate to oxaloacetate per min at pH 8.1.

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of AST / Aspartate Transaminase ranged from 2- 100 U/l. The mean MDD was 2 U/l