# Aspartate Aminotransferase Colorimetric Activity Assay Kit

Item No. 701640

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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# GENERAL INFORMATION

## Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening the kit, remove components and store as stated below.

| Item Number | Item | Quantity | Storage |
|---|---|---|---|
| 701641 | AST Assay Buffer (10X) | 1 vial/5 ml | -20°C |
| 701642 | AST Substrate | 1 vial/20 ml | -20°C |
| 701643 | AST Cofactor | 2 vials | -20°C |
| 701644 | AST Initiator | 1 vial/3 ml | -20°C |
| 701645 | AST Positive Control | 1 vial | -20°C |
| 400014 | 96-Well Solid Plate (Colorimetric Assay) | 1 plate | RT |
| 400012 | 96-Well Cover Sheet | 1 cover | RT |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

## If You Have Problems

**Technical Service Contact Information**


**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Fax:** 734-971-3640

**Email:** techserv@caymanchem.com


In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 340 nm
2. Adjustable pipettes and a multichannel or repeating pipette
3. A source of pure water; glass-distilled water or HPLC-grade water is acceptable *NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000)*

# INTRODUCTION

## Background

Aspartate aminotransferase (AST) is a homodimeric pyridoxal phosphate-dependent enzyme involved in amino acid metabolism.[1] It mediates the reversible transamination between aspartate and α-ketoglutarate to form oxaloacetate and glutamate. This interconversion provides a route for transporting oxaloacetate across the mitochondrial membrane, in the form of aspartate, into the cytosol for use in gluconeogenesis.

AST is widely distributed in many tissues but found in the greatest abundance in the liver, cardiac and skeletal muscle, kidneys, and brain.[2] Serum AST levels are typically low but increase during disease states allowing it to be used as a general biomarker for liver or other organ damage. Because AST is expressed in a wide variety of tissues, it is not as specific as alanine aminotransferase (ALT) as a biomarker of liver damage, however, AST can be used in conjunction with other factors to assess liver injury. AST and type IV collagen 7S serum levels have been used together as an index to predict non-alcoholic steatohepatitis (NASH) in patients with non-alcoholic fatty liver disease (NAFLD).[3] The AST-to-platelet ratio index (APRI) has been used to predict liver fibrosis in healthy adults and to assess it in a variety of disease states, including chronic hepatitis B virus infection.[4,5] In addition, the ratio of AST to ALT in serum has been used as an indicator of alcoholic liver disease, with a high ratio being indicative of disease.[6]



## About This Assay

Cayman's Aspartate Aminotransferase Colorimetric Activity Assay Kit provides a convenient method of detecting AST activity in serum, plasma, tissue samples, and cell lysates. Measurement of the AST activity is carried out by monitoring the rate of NADH oxidation in a coupled reaction system employing malate dehydrogenase (MDH) (see Figure 1). The oxidation of NADH to $NAD^+$ is accompanied by a decrease in absorbance at 340 nm. Under circumstances in which the AST activity rate is limiting, the rate of decrease is directly proportional to the AST activity in the sample. Lactate dehydrogenase (LDH) is added to prevent interference from endogenous pyruvate normally present in serum.

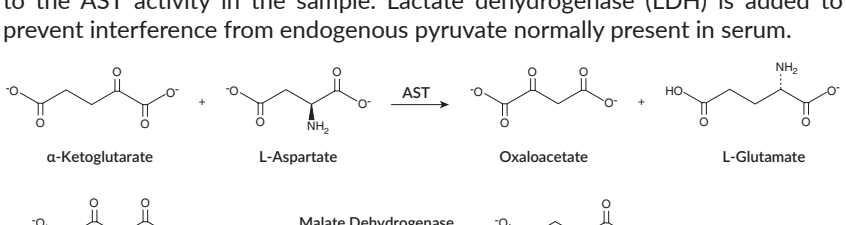


**Figure 1. Assay scheme**

# PRE-ASSAY PREPARATION

## Reagent Preparation

1. **AST Assay Buffer (10X) - (Item No. 701641)**

   Mix 4 ml of AST Assay Buffer (10X) with 36 ml of HPLC-grade water. This final diluted assay buffer, Assay Buffer (1X) (100 mM Tris-HCl, pH 7.8, 10 mM sodium bicarbonate, 0.1 mM pyridoxal-5'-phosphate, 0.01% sodium azide), should be used in the assay and for reconstituting the cofactor and positive control. This diluted buffer is stable for six months when stored at 4°C.

2. **AST Substrate - (Item No. 701642)**

   This vial contains 20 ml of an L-aspartate solution. The reagent is ready to use as supplied.

3. **AST Cofactor - (Item No. 701643)**

   This vial contains a lyophilized powder of NADH, LDH, and MDH. Immediately prior to assaying, reconstitute the entire contents of one vial with 1.5 ml of Assay Buffer (1X). This will make a sufficient volume of AST Cofactor to assay 75 wells. Reconstitute two vials if running a full plate. The reconstituted AST cofactor is stable for two weeks when stored at -20°C.

4. **AST Initiator - (Item No. 701644)**

   This vial contains 3 ml of 150 mM α-ketoglutarate. The reagent is ready to use as supplied. This reagent is stable for six months when stored at -20°C and five days when stored at 4°C.

5. **AST Positive Control - (Item No. 701645)**

   This vial contains lyophilized AST from porcine heart. Reconstitute the contents of the vial with 2 ml of Assay Buffer (1X).

## Sample Preparation

### Plasma

Typically, normal human plasma has AST concentrations in the range of 8-40 U/L.[7]

1. Collect blood using an anticoagulant such as heparin or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, store at -80°C. Avoid repeated freeze/thaw cycles.
3. Plasma does not need to be diluted before assaying.

### Serum

Typically, normal human serum has AST concentrations in the range of 8-40 U/L.[8]

1. Collect blood without using an anticoagulant such as heparin or citrate.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, store at -80°C. Avoid repeated freeze/thaw cycles.
4. Serum does not need to be diluted before assaying.

### Tissue Homogenate

1. Prior to dissection, rinse the tissue with a PBS solution, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 5-10 ml of cold buffer (*e.g.*, 100 mM Tris, pH 7.8) per gram of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -20°C.

*NOTE: If the decrease in the rate of absorbance at a wavelength of 340 nm ($A_{340}$) is greater than 0.4 absorbance units/min., dilution of the sample with Assay Buffer (1X) will be necessary to fall within the linear range of the assay.*

### Cell Lysate

1. Collect cells (~5 x $10^6$) by centrifugation (*i.e.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, use a rubber policeman instead of proteolytic enzymes.
2. Homogenize the cell pellet in 0.5-1.0 ml cold buffer (*i.e.*, 100 mM Tris, pH 7.5, 1 mM EDTA).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, freeze the sample at -80°C.

*NOTE: If the rate of $A_{340}$ decrease is greater than 0.4 absorbance units/min, dilution of the sample with Assay Buffer (1X) will be necessary to fall within the linear range of the assay.*

# ASSAY PROTOCOL

## Plate Set Up

There is no specific pattern for using the wells on the plate. It is recommended that three wells be designated for the AST Positive Control. It is suggested that each sample be assayed in triplicate and that the contents of each well are recorded on the template sheet provided on page 21. A typical layout of samples to be measured in triplicate is given below.

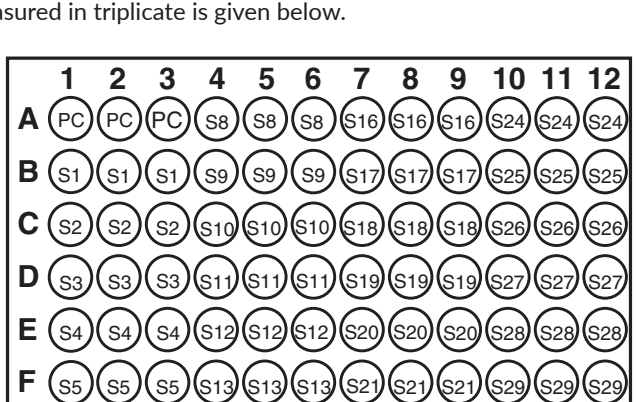


**Figure 2. Sample plate format**

**Pipetting Hints**

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

**General Information**

- The final volume of the assay is 210 µl per well.
- Use the diluted assay buffer in the assay.
- This assay is very sensitive to changes in temperature. For best results, all reagents should be equilibrated to 37°C before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- 31 samples can be assayed in triplicate or 46 in duplicate.
- The assay is performed at 37°C.
- Monitor the absorbance at 340 nm.

## Performing the Assay

1. **Positive Control Wells:** Add 150 µl of AST Substrate, 20 µl of AST Cofactor, and 20 µl of AST Positive Control to the designated wells on the plate (see **Sample plate format**, Figure 2, page 12).
2. **Sample Wells:** Add 150 µl of AST Substrate, 20 µl of AST Cofactor, and 20 µl of sample to the designated wells on the plate.
3. **Sample Background Wells (optional):** The background activity is typically insignificant in the evaluation of AST activity in a sample. However, if desired, a background value can be obtained for each sample. For each sample being assayed, add 150 µl of Assay Buffer (1X), 20 µl of AST Cofactor, and 20 µl of sample to the designated wells on the plate.

| Well | AST Substrate | Assay Buffer (1X) | AST Cofactor | AST Positive Control | Sample |
|---|---|---|---|---|---|
| Positive Control | 150 µl | - | 20 µl | 20 µl | - |
| Sample | 150 µl | - | 20 µl | - | 20 µl |
| Sample Background | - | 150 µl | 20 µl | - | 20 µl |

**Table 1. Pipetting summary**

4. Cover plate and incubate at 37°C for 15 minutes.
5. Remove the plate cover and quickly initiate the reactions by adding 20 µl of AST Initiator to all the wells being used.
6. Immediately measure the absorbance at 340 nm once every minute for 10 minutes at 37°C. If AST activity is low, continue reading for up to 60 minutes.



# ANALYSIS

## Calculations

1. Determine the change in absorbance ($\Delta A_{340}$) per minute:
   a. Plot the absorbance values as a function of time to obtain the slope (rate) of the linear protion of the curve (a graph is shown using porcine heart AST, see Figure 3, page 16).

   **OR**

   b. Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation. *Use the absolute value.

$$\Delta A_{340}/\text{min.} = \frac{*|\, A_{340}\,(\text{Time 2}) - A_{340}\,(\text{Time 1})\,|}{\text{Time 2 (min.)} - \text{Time 1 (min.)}}$$

*Use the absolute value.

2. If running background wells, determine the rate of $\Delta A_{340}$ for the background and subtract this rate from that of the sample wells. The reaction rate at 340 nm can be determined using the NADH extinction coefficient of 4.11 $mM^{-1}$.* One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 µmol of NADH to $NAD^+$ per minute at 37°C.
3. Use the following formula to calculate the AST activity.

$$\text{AST activity (U/ml)} = \left[\frac{\Delta A_{340}/\text{min} \times 0.21\ \text{ml}}{4.11\ mM^{-1} \times 0.02\ \text{ml}}\right] \times \text{Sample dilution}$$

*The actual extinction coefficient for NADH at 340 nm is 6.22 $mM^{-1}cm^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.66 cm).

*NOTE: To convert to SI units (IU) or nKat/ml, multiply U/ml by a factor of 16.67. To convert to U/L, multiply U/ml by 1,000.*

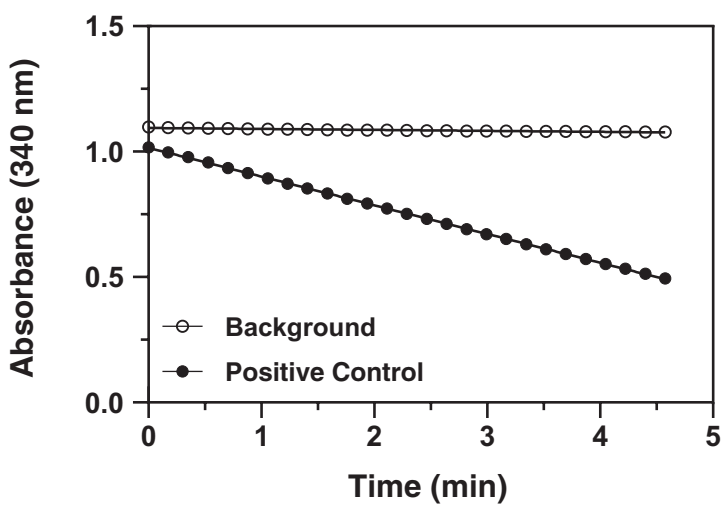


Figure 3. Activity of porcine heart AST

**Spike and Recovery**

Human serum samples were spiked with AST and analyzed using the Aspartate Aminotransferase Colorimetric Activity Assay Kit. The results are shown below.

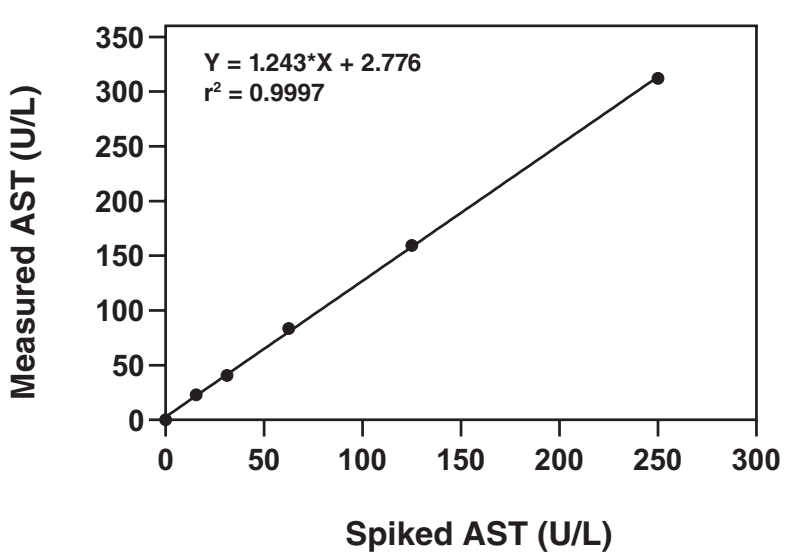


Figure 4. Spike and recovery in human serum

## Performance Characteristics

**Precision:**

When a series of seven AST measurements were performed on the same day under the same experimental conditions, the intra-assay coefficient of variation was 4.1%. When a series of AST measurements were performed on ten different days under the same experimental conditions, the inter-assay coefficient of variation was 5.4%.

**Sensitivity:**

Samples containing AST activity between 0.011-0.8 U/ml can be assayed without further dilution or concentration.



# RESOURCES

## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates/triplicates | A. Poor pipetting/technique<br>B. Bubble in the well(s) | A. Be careful not to splash the contents of the wells<br>B. Carefully tap the side of the plate with your finger to remove bubbles |
| No decrease in absorbance but has a high initial absorbance (~0.9) | A. Sample was not added to the wells<br>B. AST activity is too low to detect | A. Make sure to add all the components to the wells and re-assay<br>B. Concentrate the sample with an Amicon concentrator with a molecular weight cut-off of 10 kDa and re-assay |
| No decrease in absorbance but has a low initial absorbance (<0.3) | Little or no cofactor was added to the well in question | Make sure to add all the components to the wells and re-assay |

## References


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2. Pratt, D.S. and Kaplan, M.M. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N. Engl. J. Med.* **342(17)**, 1266-1271 (2000).
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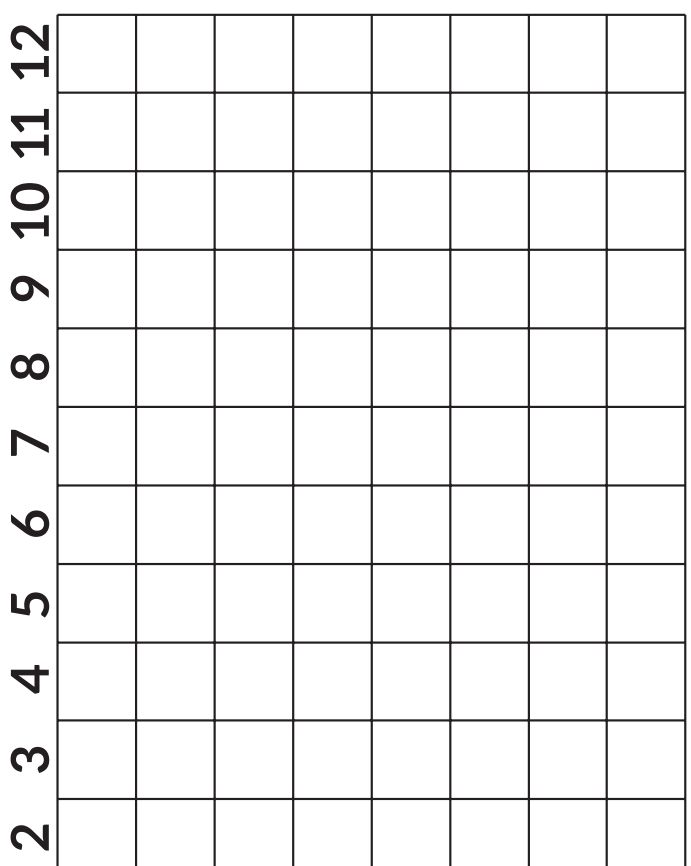
4. Unalp-Arida, A. and Ruhl, C.E. Liver fibrosis scores predict liver disease mortality in the United States population. *Hepatology* **66(1)**, 84-95 (2018).
5. Xiao, G., Yang, J., and Yan, L. Comparison of diagnostic accuracy of aspartate aminotransferase to platelet ratio index and fibrosis-4 index for detecting liver fibrosis in adult patients with chronic hepatitis B virus infection: A systemic review and meta-analysis. *Hepatology* **61(1)**, 292-302 (2015).
6. Torruellas, C., French, S.W., Medici, V. Diagnosis of alcoholic liver disease. *World J. Gastroenterol* **20(33)**, 11684-11699 (2014).
7. Siest, G., Schiele, F., Galteau, M.M., *et al.* Asparate aminotransferase and alanine aminotransferase activities in plasma: Statistical distributions, individual variations, and reference values. *Clin. Chem.* **21(8)**, 1077-1087 (1975).
8. Kim, K.-N., Joo, J., Sung, H.K., *et al.* Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: A large-scale national health screening cohort study. *BMJ Open* **9(5)**, e026965 (2019).

## NOTES



## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.