MA-0120

Aspartate Aminotransferase Activity Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Background Information:

Aspartate aminotransferase (AST), also known as Glutamate-oxaloacetate transaminase (GOT) is a pyridoxal phosphate dependent transaminase. AST exists as two isozymes, cytosolic and mitochondrial with different blood half-lives. Due to its central role in amino acid metabolism, AST is a biomarker for liver function. AST activity, being significant in several organs other than liver, is a broader clinical indicator. AST catalyzes the reaction Aspartate + α-Ketoglutarate ⇌ Oxaloacetate + Glutamate. AkrivisBio's AST Activity Assay is a simple, sensitive way to measure AST activity from a wide range of biological samples with a detection limit of less than 0.02 mU per well.

Assay Principle:

1 - Aspartate + α-ketoglutarate are converted to oxaloacetate + glutamate
2 - Glutamate is oxidized by glutamate dehydrogenase forming NADH in the process
3 - NADH reduces WST from a nearly colorless tetrazolium to a highly colored formazan

Assay Components:

Assay Buffer	25 ml	WM	MA-0120A
Glutamate Dehydrogenase	lyoph	Green	MA-0120B
WST Reagent	lyoph	Red	MA-0120C
Aspartate/α-ketoglutarate	lyoph	Orange	MA-0120D
Glutamate Standard	100 µl	Yellow	MA-0120E
AST Positive Control	lyoph	Blue	MA-0120F

Storage and Handling:

Store the kit at -20°C. Allow all components to warm to room temperature before use. Centrifuge vials for a few seconds before opening. Once reconstituted, all components should be aliquoted into convenient portions and stored at -20°C to avoid multiple freeze/thaw cycles.
Glutamate dehydrogenase: Reconstitute with 220 µl DI H_2O.
WST Reagent: Reconstitute with 220 µl DI H_2O.
Aspartate/α-ketoglutarate: Reconstitute with 1.1 ml assay buffer.
AST Positive Control: Reconstitute with 100 µl DI H_2O.

Assay Protocol:

1. Standard Curve: Transfer 10 µl of Glutamate Standard to a 1 ml tube. Add 490 µl Assay Buffer and mix. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer to give 0 – 2 – 4 – 6 – 8 – 10 nmol per well of Glutamate Standard.

2. Sample Preparation: Homogenize tissue (10 mg) or cells (10^6) with 200 µl of ice-cold Assay Buffer. Centrifuge at 16,000 X g for 5 minutes to remove particulates and cell debris. Serum can be used directly in the assay. If the activity is very high dilute a serum sample with Assay Buffer. Transfer 10-25 µl to wells of a 96 well plate. Adjust all well volumes to 50 µl. All OD values used to determine enzyme activity must be within the range of the standard curve. If the activity is too fast to allow for use of OD values within the standard curve range, dilute the sample and run it again.

3. Positive Control (Optional): Take 5 µl and add 50 µl of Assay Buffer. Transfer 2 - 10 µl to wells. Adjust well volume to 50 µl.

4. Initiate Reaction: Each well will require 100 µl of Reaction Mix. Prepare sufficient Reaction Mix for the number of standards and samples to be run. Mix enough reagent for the number of assays to be performed. For each well, prepare:

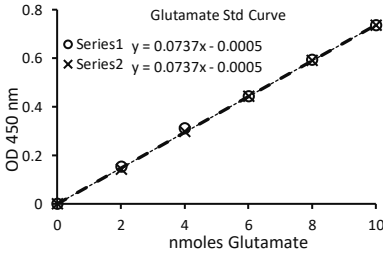
	Reaction Mix
Assay Buffer	86 µl
Glutamate Dehydrogenase	2 µl
WST Reagent	2 µl
Aspartate/α-ketoglutarate	10 µl

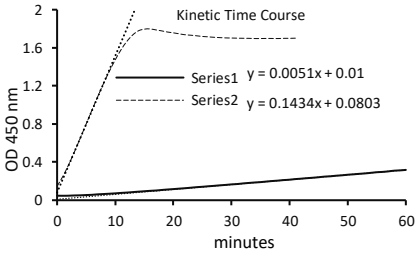
Add 100 µl of the Reaction Mix to each well containing Samples, Standards or Positive Controls.

5. Measurement: Monitor the reaction in kinetic mode at 37°C for 1 hour at 450 nm. If AST activity is low it can be monitored for a longer period of time. There is an initial lag phase while the reactants warm to 37°C. Determination of reaction rates must wait until this lag period is over.

6. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values	
0	0.1318	0.1297	0	0
2	0.2848	0.2731	0.1530	0.1434
4	0.4431	0.4272	0.3113	0.2975
6	0.5743	0.5722	0.4425	0.4425
8	0.7254	0.7201	0.5936	0.5904
10	0.8679	0.8649	0.7361	0.7352





The kinetic time course shows the results for 2 and 0.05 mU of AST activity in the well. The reaction rate for 2 mU is too fast and there is not really a good linear portion. It needs to be diluted and rerun. The 0.05 mU sample shows a lag that persists for at least 25 minutes before the reaction becomes linear.

7. Calculation: Subtract the value of the 0 glutamate standard from all of the other standards. Determine the slope of the standard curve. This defines the OD/nmole obtained from the experiment. Determine the rate of enzyme activity (Δ OD/ Δ t (minutes)by finding a linear portion of the activity curve and determining the change in OD (ΔOD) and the time period over which the change in OD occurred (Δ t). do the same for the 0 glutamate standard. There will be a slight drift due to non-enzymatic factors. Subtract the rate of the 0 glutamate standard from the enzyme rate to get a corrected enzyme rate. Divide the corrected rate of enzyme activity by the slope of the standard curve to get enzyme rate in nmoles/minute (mU)in the well.
To convert back to activity in the original sample:
Enzyme rate in well (determined above) X (total volume of extract (from step **2.** above) / volume added to well) = Total Activity from Sample
Total Activity from Sample / mg of raw sample (µl of fluid sample before dilution) = activity as (mU)/mg or µl of sample

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