MA-0159

L-Asparaginase Activity Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia..Asparaginase does not occur naturally in humans but is found in bacteria, plants and some animals. Since asparagine is an essential amino acid in certain cell types, including some cancers, controlling the circulating levels of asparagine is a way of inhibiting protein synthesis needed for rapid growth. AkrivisBio's Asparaginase Activity Assay provides a simple, sensitive means of measuring asparaginase activity in biological samples.

Assay Principle:

1 – Asparaginase hydrolyzes asparagine to aspartate and ammonia.
2 – Aspartate is used by glutamate oxaloacetate transaminase to form pyruvate.
3 – Pyruvate is oxidized with the formation of hydrogen peroxide.
4 – Peroxidase utilizes the hydrogen peroxide to convert ADHP to resorufin with a large increase in color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0159A
ADHP Solution	200 µl	Red	MA-0159B
Asparagine	lyoph	Orange	MA-0159C
GOT Enzyme Mix	lyoph	Green	MA-0159D
Oxidation Enzyme Mix	lyoph	Purple	MA-0159E
Asparaginase Positive Control	lyoph	Blue	MA-0159F
Aspartate Standard 40 mM	100 µl	Yellow	MA-0159G

Storage and Handling Considerations:

Store unopened assay at -20°C. Centrifuge all small vials briefly, prior to opening. Bring all assay components to room temperature before using.
Assay Buffer: Ready to use as supplied.
ADHP Solution: Ready to use as supplied. Store at -20°C. Light and moisture sensitive.
Asparagine: Reconstitute with 440 µl DI H_2O. Store at -20°C.
GOT Enzyme Mix, Oxidation Enzyme Mix: Reconstitute with 220 µl Assay Buffer. Aliquot to convenient portions and store at -20°C.
Asparaginase Positive Control: Reconstitute with 100 µl Assay Buffer. Aliquot to convenient portions and store at -20°C.
Aspartate Standard: Ready to use as supplied. Store at -20°C**.**

Assay Protocol:

1. Standard Curve Preparation:
Absorbance-based Assay*:* Dilute the Aspartate Standard to 0.4 mM by adding 10 µl of the Aspartate Standard stock to 990 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the diluted standard to a series of wells in a 96-well plate. Adjust all wells to 50 µl with Assay Buffer, giving 0, 2, 4, 6, 8, and 10 nmol respectively of the Aspartate Standard.
Fluorescence-based Assay: Dilute the standard as for absorbance, then further dilute the 0.4 mM solution again to 40 µM by adding 20 µl to 180 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to wells of a 96-well plate, giving 0, 200, 400, 600, 800, 1000 pmol, respectively of the Aspartate Standard.

2. Sample Preparation:
Clarify biological fluids (plasma, serum) by centrifugation at 16,000 x g for 5 min to remove insoluble materials. Homogenize tissue (10 mg) or cells (10^6) using 100 µl ice cold Assay Buffer. Centrifuge at 16,000 x g for 10 min at 4°C and transfer the supernatant to a fresh tube.
Add 2-10 µl of test samples to wells to a 96-well plate and adjust all wells to 50 µl with Assay Buffer.
Notes: Background interference is usually negligible in absorbance-based assays. Fluorescence based assays should be run as pairs with one of the pair used as a background control, omitting asparagine from the assay, showing signal not due to asparagine hydrolysis. Test sample readings should be within the range of the standard curve. Any which exceed that range should be diluted and rerun.

3. Positive Control: Add 2 µl of the Asparaginase Positive Control to a well(s) and adjust the volume to 50 µl/well with Assay Buffer.

4. Initiate Reaction: Each Standard, Test sample and Positive Control well will require 50 µl of Rection Mix. Prepare sufficient material for the total number of wells to be analyzed, containing:

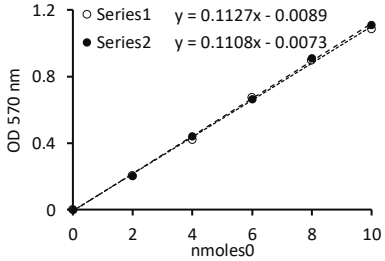
	Absorbance Reaction Mix	Fluorescence Reaction Mix	Background Control Mix
Assay Buffer	40 µl	41.5 µl	44 µl
Asparagine	4 µl	4 µl	—---
GOT Enzyme Mix	2 µl	2 µl	2 µl
Oxidation Enzyme Mix	2 µl	2 µl	2 µl
ADHP Solution	2 µl	0.5 µl	0.5 µl

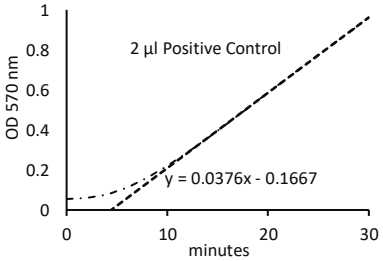
Add 50 µl of Reaction Mix to all Sample, Standard and Positive Control wells. Add 50 µl of Background Control Mix to any Background Control wells.

5. Measurement: Monitor reaction progress using absorbance (570 nm) or fluorescence (Excitation 535; Emission 587 nm) in kinetic mode at 25°C for 30-60 min. Beyond about 0.7 OD, reaction progress will slow due to depletion of substrate.

6. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0485	0.0516	0	0
2	0.254	0.2553	0.2055	0.2037
4	0.4675	0.4930	0.4190	0.4414
6	0.7221	0.7146	0.6736	0.6630
8	0.9452	0.9610	0.8967	0.9094
10	1.1341	1.1617	1.0856	1.1101





7. **Calculations: S**ubtract the zero standard reading from all of the standard readings. Plot the Standard Curve. Determine the slope of the Standard Curve. Subtract Background Control readings from the paired Test sample. If no Background Controls have been run, subtract the zero standard from the test sample readings. There will be a 10-15 minute lag phase before test samples show linear enzyme activity. Determine the slope (OD/min or RFU/min) of the test samples in the linear portion of the reaction. Divide the slope of the test samples by the slope of the Standard Curve to convert to nmoles or pmoles per minute. In the figure shown the slope of the Positive Control is 0.0376 OD/min. Dividing by the slope of the Standard Curve (avg 0.1117) gives 0.3366 or about 0.33 nmol/min or ~330 µU of enzyme activity in 2 µl.

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