Amplite® Fluorimetric Asparaginase Activity Assay Kit

Catalog number: 11799
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11799)
Component A: Amplite® Red Substrate	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B1: Enzyme Mix 1	Freeze (< -15 °C)	1 Bottle (lyophilized powder)
Component B2: Enzyme Mix 2	Freeze (< -15 °C)	1 Vial (lyophilized powder)
Component C: Assay Buffer	Freeze (< -15 °C)	1 Bottle (5 mL)
Component D: Conversion Mix	Freeze (< -15 °C)	1 Vial (lyophilized powder)
Component E: Aspartate Standard	Freeze (< -15 °C)	1 Vial
Component F: Asparaginase Positive Control	Freeze (< -15 °C)	1 Vial (lyophilized powder)
Component G: DMSO	Freeze (< -15 °C)	1 Vial (100 µL)

OVERVIEW

The Amplite® Fluorimetric Asparaginase Activity Assay Kit provides a simple and straightforward procedure for measuring Asparaginase activity in a variety of biological samples. Asparaginase activity is determined by a coupled enzyme assay, which results in the formation of a fluorogenic product with Ex/Em=540/590nm. The amount of the fluorogenic product formed is proportional to the aspartate generated by asparaginase enzyme. 1 unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. Asparaginase is an essential enzyme that catalyzes the hydrolysis of the non-essential amino acid asparagine to aspartate and ammonia. It plays a crucial role in cellular functions, particularly in hematopoietic cells which rely on exogenous asparagine for protein synthesis. Asparaginase is found in plants, microorganisms, and certain animals, but does not occur naturally in humans, making it a valuable therapeutic agent in medicine and an essential tool in various industries. Asparaginase is used to treat acute lymphocytic leukemia (ALL) by starving tumor cells of needed nutrients and slowing tumor cell growth. Depletion of circulating asparagine by asparaginase induces cell cycle arrest and apoptosis in malignant cells, offering a targeted approach to cancer treatment. Beyond its medical applications, asparaginase is also used in the food industry to reduce the formation of acrylamide, a potentially carcinogenic compound, in starchy and fried foods. The versatility of this enzyme extends to various research fields, including biotechnology and biochemistry.

AT A GLANCE

Important Note

Thaw all the kit components at room temperature before starting the experiment.

Protocol Summary

1. Prepare test samples and serially dilute Aspartate or Asparaginase standards (50 µL).
2. Add the Asparaginase working solution (50 µL).
3. Incubate at 37 °C for 10-30 minutes.
4. Monitor the fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm).

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	570 nm
Emission	590 nm
Excitation	540 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Amplite® Red Substrate Stock Solution (100X)

1. To prepare a 100X Amplite® Red Substrate stock solution, add 60 µL of DMSO (Component G) to the vial of Amplite® Red Substrate (Component A).

Enzyme Mix 2 Stock Solution (50X)

1. To prepare a 50X Enzyme Mix 2 stock solution, add 100 µL of ddH2O to the Enzyme Mix 2 vial (Component B2).

Conversion Mix Stock Solution (100X)

1. To prepare a 100X Conversion Mix stock solution, add 50 µL of ddH2O to the Conversion Mix vial (Component D).

Aspartate Standard Solution (10mM)

1. To prepare at 10 mM aspartate standard solution, add 100 µL of ddH2O to the Aspartate Standard vial (Component E).

Asparaginase Positive Control Stock Solution

1. Reconstitute Asparaginase Positive Control (Component F) with 100 µL of ddH2O, and mix well. Aliquot the solution and store it at -20 °C.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11799

Aspartate Standard

Add 12 µL of a 10 mM aspartate standard to 288 µL of 1X PBS buffer to prepare a 400 µM aspartate solution (STD7). Next, perform 1:2 serial dilutions in 1X PBS buffer to obtain the serially diluted aspartate standards (STD6-STD1).

PREPARATION OF WORKING SOLUTION

Asparaginase Working Solution

1. Add 5 mL of Assay Buffer (Component C) to the Enzyme Mix 1 bottle (Component B1) and mix well.

2. Add 100 µL of Enzyme Mix 2 stock solution to the same bottle and mix well.

3. Add 50 µL of the 100X Conversion Mix stock solution to the same bottle and mix well.

4. Add 50 µL of the Amplite® Red Substrate stock solution to the same bottle and mix well.

   Note: The working solution is not stable, use it promptly, and avoid direct exposure to light.

   Note: Alternatively, one can make a 50X Enzyme Mix 1 stock solution by adding 100 µL of ddH2O into the bottle of Enzyme Mix 1 (Component B1) and then prepare the Asparaginase working solution by mixing the Enzyme Mix 1 stock solution with other components listed above in the 'Asparaginase Working Solution' proportionally.

Asparaginase Positive Control

1. To prepare one or more Asparaginase positive controls alongside the test sample, dilute the Asparaginase Positive Control stock solution using a dilution factor between 100 and 1000 times in 1X PBS. For example, for a 250-fold dilution, mix 4 µL of Asparaginase Positive Control stock solution with 996 µL of 1X PBS.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Aspartate standards and test samples in a 96-well solid black microplate. (STD = Aspartate Standards (STD1-STD7, 6.25 to 400 µM), BL = Blank Control, TS = Test Samples)

BL	BL	Positive Control	TS
STD 1	STD 1	...	...
STD 2	STD 2	...	...
STD 3	STD 3		
STD 4	STD 4		
STD 5	STD 5		
STD 6	STD 6		
STD 7	STD 7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
STD 1 - STD 7	50 µL	Serial Dilutions (6.25 to 400 µM) in PBS
BL	50 µL	PBS
Asparaginase Positive Control	50 µL	Asparaginase Positive Control in PBS
TS	50 µL	Test Sample

1. Prepare the Aspartate standards (STD 1-STD 7), blank controls (BL), Asparaginase Positive Control, and test samples (TS) according to the layout provided in Tables 1 and 2. When using a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of Asparaginase Working Solution to each well of the blank control, Asparaginase Positive Control, and test samples. If using a 384-well plate, add 25 µL of Asparaginase Working Solution to each well instead.

3. Incubate at 37 °C for 10-30 minutes, protected from light.

4. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 540 nm/ 590 nm (Cutoff = 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The fluorescence reading in the blank wells (containing only PBS) is used as a control. Subtract this control value from the readings of the wells containing the standards, Asparaginase positive controls, and test samples. The standard curve of Aspartate is shown in Figure 1. To calculate the Aspartate concentrations in the samples according to the standard curve, use the Online Linear Regression Calculator, which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

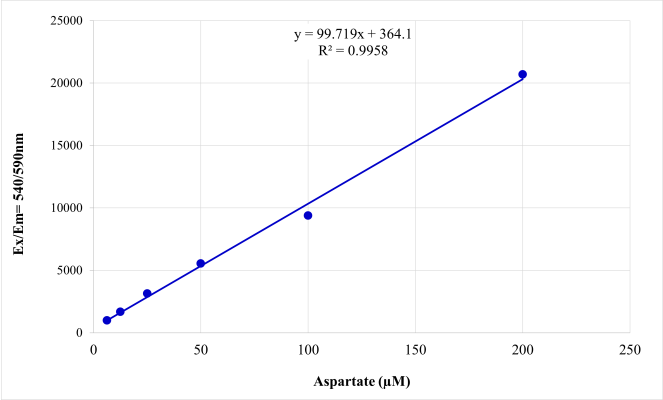

Figure 1. Aspartate dose response was measured using the Amplite® Fluorimetric Asparaginase Activity Assay Kit on a 96-well clear bottom black solid microplate using a fluorescence microplate reader (Ex/Em = 540/590, Cutoff = 570 nm).

Data Analysis Example

Calculate Asparaginase Activity

Asparaginase Positive Control (250X dilution) Signal	21709
Background	1231
After BG Correction	20478
Aspartate Generated (µM)	201.706
Time (min)	20
Activity (mU/mL)	10.085

Note:

1. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute
2. nmole/min/mL = µM/min = mU/mL

DISCLAIMER

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