Amplite® Colorimetric Asparaginase Activity Assay Kit

Catalog number: 11800
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 11800)
Component A: Amplite® Red Substrate	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B1: Enzyme Mix1	Freeze (< -15 °C)	1 Bottle
Component B2: Enzyme Mix 2	Freeze (< -15 °C)	1 Bottle
Component C: Assay Buffer	Freeze (< -15 °C)	1 Bottle (5 mL)
Component D: Conversion Mix	Freeze (< -15 °C)	1 Vial
Component E: Aspartate Standard	Freeze (< -15 °C)	1 Vial
Component F: Asparaginase Positive Control	Freeze (< -15 °C)	1 Vial
Component G: DMSO	Freeze (< -15 °C)	1 Vial (100 µL)

OVERVIEW

The Amplite® Colorimetric 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 colored product with absorbance at 570 nm. The amount of the colored product and the absorbance value is proportional to the aspartate generated by the asparaginase enzyme. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. Asparaginase is an essential enzyme catalyzing the hydrolysis of 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.

The Amplite® Colorimetric 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 colored product with absorbance at 575 nm. The amount of the colored product and the absorbance value is proportional to the aspartate generated by the asparaginase enzyme. One unit (U) is the amount of enzyme that catalyzes the reaction of 1 µmol of substrate per minute. Asparaginase is an essential enzyme catalyzing the hydrolysis of 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

Protocol Summary

1. Prepare the test samples and the serially diluted Aspartate standards (50 µL).
2. Add the Asparaginase working solution (50 µL).
3. Incubate for 10-30 minutes at 37 °C.
4. Measure the absorbance at 570 nm.

Important Note

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570 nm
Recommended plate	Clear bottom

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

   Note: The solution can be stored at -20°C. Avoid repeated freeze/thaw cycles.

Asparaginase Positive Control Stock Solution

1. Reconstitute the Asparaginase Positive Control (Component F) with 100 µL of ddH2O. Mix well by pipetting and store at -20 °C.

   Note: Must be used within 2 months of reconstitution. Avoid freeze/thaw cycles.

Aspartate Standard Solution (10 mM)

1. To prepare a 10 mM Aspartate Standard stock solution, add 100 µL of ddH2O to the vial containing the Aspartate Standard (Component E), and mix well.

   Note: The solution can be aliquoted and stored at -20°C. Avoid freeze/thaw cycles.

Conversion Mix Stock Solution (100X)

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

   Note: The solution can be aliquoted and stored at -20°C. Avoid freeze/thaw cycles.

Enzyme Mix 2 Stock Solution (50X)

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

   Note: The solution can be aliquoted and stored at -20°C. Avoid freeze/thaw cycles.

PREPARATION OF STANDARD SOLUTIONS

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11800

Aspartate Standard
Add 12 µL of the 10 mM aspartate standard solution to 288 µL of PBS buffer to make a 400 µM aspartate solution (STD7). Then, perform 1:2 serial dilutions with 1X PBS Buffer to create a series of diluted aspartate standards (STD6 to STD1).

PREPARATION OF WORKING SOLUTION

Asparaginase Working Solution

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

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

3. Transfer 50 µL of the Conversion Mix stock solution and 50 µL of the 100X Amplite® Red Substrate stock solution to the same bottle, and mix well.

   Note: This Asparaginase working solution should be freshly prepared before each experiment and protected from light. Due to its instability, it should be used immediately after preparation.

   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.

SAMPLE EXPERIMENTAL PROTOCOL

Asparaginase Positive Control

1. Prepare one or more Asparaginase positive control samples along with the test sample. The recommended dilution factor is 100-1000 fold in 1X PBS. For example, for a 250-fold dilution, mix 4 µL of the Asparaginase stock solution with 996 µL of PBS.

Table 1. Layout of Aspartate standards and test samples in a 96-well clear bottom microplate. (STD = Aspartate Standards (STD1-STD7, 6.25-400 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 Aspartate standards (STD1-7), blank controls (BL), Asparaginase Positive Control, and test samples (TS) as outlined in Tables 1 and 2. For 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 containing the Aspartate standard, blank control, Asparaginase Positive Control, and test samples. For a 384-well plate, add 25 µL of the Asparaginase Working Solution to each well instead.

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

4. Monitor the absorbance intensity with an absorbance microplate reader at 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The absorbance reading from the blank wells (containing only PBS) is used as a control and subtracted from the readings of wells containing Aspartate standards, Asparaginase positive controls, and test samples. Figure 1 shows the standard curve for Aspartate. To determine the Aspartate concentrations in your samples using this standard curve, we recommend using the Online Linear Regression Calculator available at:

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

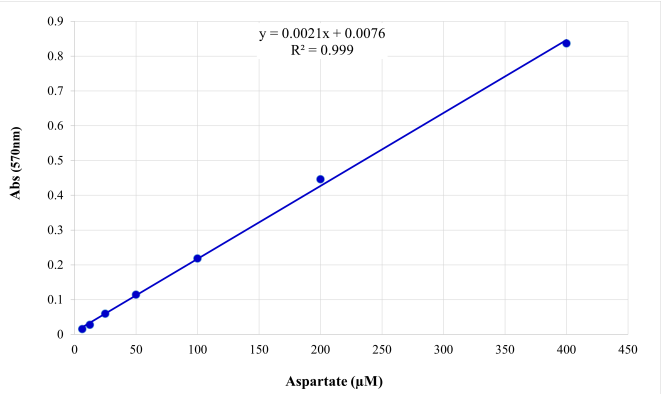

Figure 1. Aspartate dose responses was measured using the Amplite® Colorimetric Asparaginase Activity Assay Kit on a 96-well clear bottom microplate with a ClarioStar microplate reader (BMG) at 570 nm.

Data Analysis Example

Calculate Asparaginase activity

Asparaginase Positive Control (250X dilution) Signal	0.582
Background	0.144
After BG Correction	0.438
Aspartate Generated (µM)	204.952
Time (min)	20
Activity (mU/mL)	10.25

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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