Asparaginase Assay Kit

Catalog Number KA7481

100 assays

Version: 01

Intended for research use only

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Introduction

Background

ASPARAGINASE (EC 3.5.1.1) is a key enzyme in the metabolism of asparagine. Its principal use is as treatment for acute lymphoblastic leukemia and lymphoblastic lymphoma where the asparagine is an essential amino acid. Asparaginase Assay Kit provides a convenient fluorimetric method to measure asparaginase activity in biological samples. In this assay, o-phthalaldehyde reacts with liberated ammonia where the increase in fluorescence at $\lambda_{ex/em}$ = 415/475 nm is directly proportional to enzyme activity.

✓ Applications

For quantitative determination of asparaginase activity in biological samples.

✓ Key Features

- Safe. Non-radioactive assay. Assay can be completed within 40 minutes.
- Sensitive and accurate. Linear detection range 1.1 - 300 U/L asparaginase in a 96-well plate assay.
- Convenient and high-throughput. Homogeneous "mix-incubatemeasure" type assay. Can be readily automated to assay thousands of samples per day.

General Information

Materials Supplied

List of component

Component	Amount
Assay Buffer	5 mL
20 mM Standard	50 µL
20 mM Substrate	300 µL
Detection Reagent	5 mL

Storage Instruction

The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Materials Required but Not Supplied

- ✓ Pipetting devices
- ✓ Accessories (e.g. Multi-channel pipettor)
- ✓ Black flat-bottom 96-well plates (e.g. Corning costar)
- ✓ Centrifuge tubes
- ✓ Plate reader

Precautions for Use

- ✓ Reagents are for research use only.
- ✓ Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

Reagent Preparation

Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The Working Reagent should be prepared fresh for each assay run.

✓ Enzyme Preparation

Enzyme should be prepared in an enzyme buffer, e.g. 50 mM Potassium Phosphate, pH 7.4. The following protocol is optimized for recombinant E. coli Asparaginase. If using a different enzyme, we recommend that you experimentally determine the optimal amount of enzyme to use per well.

✓ Standard Preparation

Prepare a 1 mM Premix by combining 10 µL 20 mM Standard and 190 µL Assay Buffer. Transfer 10 µL of Standard to each well plus 40 µL of Assay Buffer.

No	1 mM Premix + Assay Buffer	Vol	Nitrophenol (µM)
1	100 µL + 0 µL	100 µL	1000
2	50 µL + 50 µL	100 µL	500
3	25 µL + 75 µL	100 µL	250
4	0 µL + 100 µL	100 µL	0

Sample Preparation

✓ Serum and Plasma samples must be diluted at least 1:10.

✓ Urine must be diluted 1:50 in water prior to the assay run.

Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent (WR) to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. The assay can be run at room temperature.

1. Transfer 10 µL of each sample to separate wells of the plate.
2. Prepare enough WR for all sample wells by mixing 2.5 µL of 20 mM Substrate and 45 µL of Assay Buffer for each well.
3. Initiate the reaction by addition of 40 µL of WR. Incubate the reaction for 20 minutes at RT.

   Add 50 µL Detection Reagent to all sample wells. Tap plate to mix and incubate for 20 min. Measure fluorescence intensity at $\lambda_{ex/em}$ = 415/475 nm.

Data Analysis

Calculation of Results

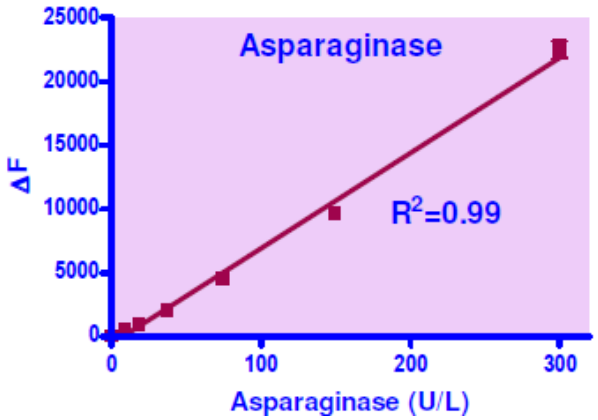
Subtract the blank value (Standard #4) from the standard values and plot ΔF against the standard concentrations. Determine the slope (µM-1) and calculate the asparaginase activity in each Sample as follows:

$$\text{Asparaginase Activity} = \frac{(F_{Sample} - F_{Blank})}{\text{Slope } (\mu M^{-1}) \times t} \times n \text{ (U/L)}$$

Where F_{Sample} and F_{Blank} are the measured fluorescence values of the sample and blank, t is the reaction time (20 min), Reaction Vol, and n is the sample dilution factor.

Unit definition: 1 Unit (U) of asparaginase will catalyze the conversion of 1 micromole of the Substrate per min at room temperature and pH 7.4.

If sample asparaginase activity exceeds 300 U/L, dilute samples in enzyme buffer and repeat the assay.



Varying amounts (300 U/L to 9.37 U/L) of highly purified E. coli Asparaginase were assayed in the presence of 1 mM Asparagine for 20 minutes at RT.

Resources

References

1. Batool, T et al. (2016) A comprehensive review on L-asparaginase and its applications. Applied Biochem. and Biotech. 178, 900-923.
2. Salzer, W. L et al. (2014). Development of asparaginase Erwinia chrysanthemi for the treatment of acute lymphoblastic leukemia. Annals of the New York Academy of Sciences, 1329, 81–92.
1. Sugawara, K, Oyama, F. (1981). Fluorgenic reaction and specific microdetermination of ammonia. J. Biochem. 89, 771–774.

Plate Layout

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