QuantiFluo™ Asparaginase Assay Kit (DASN-100)

Quantitative Fluorimetric Assay for Asparaginase Activity

DESCRIPTION

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. BioAssay Systems' DASN-100 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.

KEY FEATURES

Safe and fast. 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-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of asparaginase activity in biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 5 mL **20 mM Standard:** 50 µL
20 mM Substrate: 300 µL **Detection Reagent:** 5 mL

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

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

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.

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The *WR* 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.

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.

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	Total Volume (µL)	Std (mM)
1	100 µL + 0 µL	100 µL	1 mM
2	50 µL + 50 µL	100 µL	0.5 mM
3	25 µL + 75 µL	100 µL	0.25 mM
4	0 µL + 100 µL	100 µL	0 mM

Reaction Preparation:

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.

4. 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.

CALCULATION

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

$$\text{Asparaginase Activity} = \frac{(F_{Sample} - F_{Blank})}{\text{Slope } (mM^{-1})} \times \frac{\text{Reaction Vol } (\mu L)}{t\,(\text{min}) \times \text{Enzyme Vol } (\mu L)} \times n \text{ (U/L)}$$

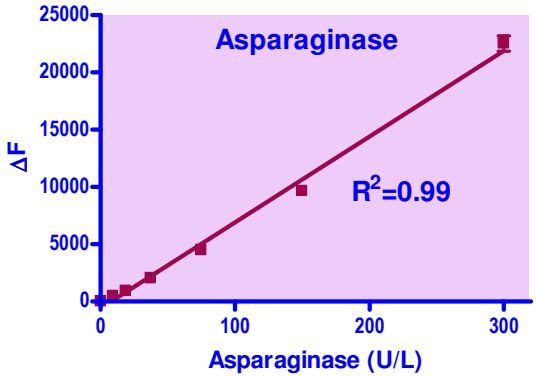
Where F_{Sample} and F_{Blank} are the measured fluorescence values of the sample and blank, t is the reaction time (20 min), Reaction Vol and Enzyme Vol are 50 µL and 10 µL, respectively, and n is the sample dilution factor.

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

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and a plate reader.



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.

LITERATURE

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.

2. Sugawara, K, Oyama, F. (1981). Fluorgenic reaction and specific microdetermination of ammonia. J. Biochem. 89, 771–774.