Asparaginase Assay Kit (BA0210)

Product Datasheet | SKU BA0210

Description

Asparaginase (EC 3.5.1.1) is a key enzyme in the metabolism of asparagine. Its principal use is as a treatment for acute lymphoblastic leukaemia and lymphoblastic lymphoma where asparagine is an essential amino acid. This 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.

Specifications

Detection Method	Fluorimetric (lambda ex/em = 415/475 nm)
Detection Range	1.1 - 300 U/L asparaginase
Sample Type	Serum, plasma, urine and other biological samples
Species Reactivity	All
Assay Time	Within 40 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Shipped at room temperature. Store all components at -20 degrees C upon receipt.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Assay Buffer	5 mL	-20 degrees C
20 mM Standard	50 uL	-20 degrees C
20 mM Substrate	300 uL	-20 degrees C
Detection Reagent	5 mL	-20 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0210	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Prior to the assay, equilibrate all components to room temperature and briefly centrifuge the tubes before opening; the Working Reagent (WR) should be prepared fresh for each assay run.
2. Prepare the enzyme in an enzyme buffer, e.g. 50 mM potassium phosphate, pH 7.4; the protocol is optimised for recombinant E. coli asparaginase and the optimal amount of a different enzyme should be determined experimentally.
3. Dilute serum and plasma samples at least 1:10 and urine 1:50 in water prior to the assay run.
4. Prepare a 1 mM Premix by combining 10 uL of 20 mM Standard and 190 uL of Assay Buffer, then dilute standards per the standard table (1000, 500, 250 and 0 uM).
5. Transfer 10 uL of each standard to a well plus 40 uL of Assay Buffer.
6. Transfer 10 uL of each sample to separate wells of the plate.

7. Prepare enough WR for all sample wells by mixing 2.5 uL of 20 mM Substrate and 45 uL of Assay Buffer for each well.
8. Initiate the reaction by adding 40 uL of WR and incubate the reaction for 20 minutes at room temperature.
9. Add 50 uL of Detection Reagent to all sample wells, tap the plate to mix and incubate for 20 minutes.
10. Measure fluorescence intensity at lambda ex/em = 415/475 nm.

Standard Preparation

No.	1 mM Premix	+ Assay Buffer	Total Volume	Std (uM)
1	100 uL	0 uL	100 uL	1000
2	50 uL	50 uL	100 uL	500
3	25 uL	75 uL	100 uL	250
4	0 uL	100 uL	100 uL	0

Calculation

Subtract the blank value (Standard #4) from the standard values and plot delta-F against the standard concentrations. Determine the slope (per uM) and calculate the asparaginase activity in each sample as: Asparaginase Activity = [(F_Sample - F_Blank) / (Slope (per uM) x t)] x n (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) and n is the sample dilution factor. Unit definition: 1 Unit (U) of asparaginase will catalyse the conversion of 1 micromole of the substrate per minute at room temperature and pH 7.4. If sample asparaginase activity exceeds 300 U/L, dilute samples in enzyme buffer and repeat the assay.

Notes

- This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent 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.
- Serum and plasma samples must be diluted at least 1:10; urine must be diluted 1:50 in water prior to the assay run.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.