Adenosine Deaminase Assay Kit (BA0209)

Product Datasheet | SKU BA0209

Description

Adenosine deaminase (EC 3.5.4.4) is a key enzyme in purine metabolism and plays an important role in the normal immune function of humans. ADA deficiency is one cause of Severe Combined Immunodeficiency (SCID), and elevated levels of ADA have been observed in patients with tuberculosis and immune-related diseases. This kit provides a convenient fluorimetric method to measure adenosine deaminase activity in biological samples. In this assay, liberated ammonia reacts with the reagents 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	0.3 - 30 U/L adenosine deaminase
Sample Type	Serum, plasma, saliva and other biological samples
Species Reactivity	All
Assay Time	Within 50 minutes
Kit Size	100 Assays
Equipment Required	Not applicable
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	4 mL	-20 degrees C
20 mM Standard	50 uL	-20 degrees C
5 mM Substrate	200 uL	-20 degrees C
Detection Reagent	5 mL	-20 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0209	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. 0.05% BSA in 200 mM potassium phosphate, pH 7.4; the protocol is optimised for adenosine deaminase from calf intestine and the optimal amount of a different enzyme should be determined experimentally.
3. Dilute serum and plasma samples at least 1:20 and saliva at least 1:50 in water prior to the assay run.
4. Prepare a 1 mM Standard Premix by combining 10 uL of 20 mM Standard and 190 uL of water, then prepare all standards according to the standard dilution table (1000, 600, 300 and 0 uM).
5. Transfer 10 uL of each standard into separate wells of a black 96-well plate.
6. Transfer 10 uL of each sample into separate wells of the plate.

7. Prepare enough WR for all assay wells by mixing 2 uL of 5 mM Substrate and 40 uL of Assay Buffer for each well.
8. Initiate the reaction by adding 40 uL of WR to all wells, tap the plate to mix and incubate for 30 minutes at room temperature.
9. Add 50 uL of Detection Reagent to all 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.	Premix	+ dH2O	Total Volume	Std (uM)
1	100 uL	0 uL	100 uL	1000
2	60 uL	40 uL	100 uL	600
3	30 uL	70 uL	100 uL	300
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 ADA activity in each sample as: ADA Activity = [(F_Sample - F_Blank) / (Slope (per uM) x t (min))] 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 (30 min) and n is the sample dilution factor. Unit definition: 1 Unit (U) of ADA will catalyse the deamination of 1 micromole of adenosine per minute at room temperature and pH 7.4. If sample ADA activity exceeds 30 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 1:20; saliva must be diluted at least 1:50 in water prior to the assay run.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.