QuantiFluo™ Adenosine Deaminase Assay Kit (DADA-100)

Quantitative Fluorimetric Assay for Adenosine Deaminase Activity

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. BioAssay Systems' DADA-100 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.

KEY FEATURES

Fast and Safe. Assay can be completed within 50 minutes. Non-radioactive assay.

Sensitive and accurate. Linear detection range is 0.3 to 30 U/L adenosine deaminase 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 adenosine deaminase enzyme activity in biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 4 mL **20 mM Standard:** 50 µL
5 mM Substrate: 200 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. 0.05% BSA in 200 mM Potassium Phosphate, pH 7.4. The following protocol is optimized for adenosine deaminase from calf intestine. 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 1:20.

Saliva must be diluted at least 1:50 in water prior to the assay run.

Standard Preparation: Prepare a 1 mM Standard Premix by combining 10 µL 20 mM Standard and 190 µL H_2O. Prepare all standards according to the table below. Transfer 10 µL of Standard to each well plus 40 µL of Assay Buffer.

No.	Premix + Assay Buffer	Total Volume (µL)	Std (µM)
1	100 µL + 0 µL	100 µL	1000
2	60 µL + 40 µL	100 µL	600
3	30 µL + 70 µL	100 µL	300
4	0 µL + 100 µL	100 µL	0

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 µL of 5 mM Substrate and 40 µL of Assay Buffer for each well.
3. Initiate the reaction by addition of 40 µL of *WR* to all sample wells. Incubate the reaction for 30 minutes at RT.
4. Add 50 µL *Detection Reagent* to all 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 (μM^{-1}) and calculate the ADA activity in each Sample as follows,

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

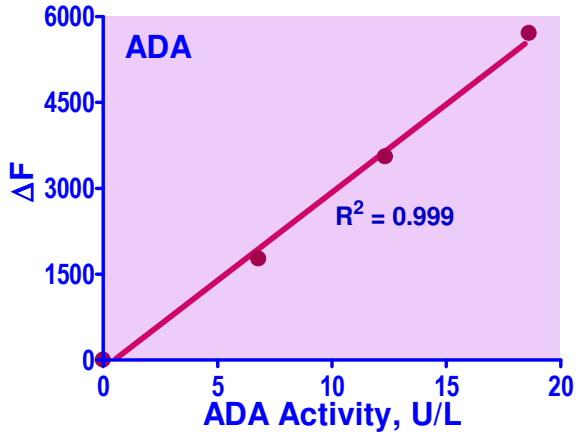
Where F_{Sample} and F_{Blank} are the measured fluorescence values of the sample and blank, t is the reaction time (30 min), and n is the sample dilution factor.

Unit definition: 1 Unit (U) of ADA will catalyze the deamination of 1 micromole of adenosine per min at room temperature and pH 7.4.

Note: If sample ADA activity exceeds 30 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 of ADA from calf intestine were assayed in the presence of 200 µM Adenosine for 30 minutes at RT.

LITERATURE

1. Xu, Z., Geng, L., Guo, L., Song, H., Pan, J., Shen, H., & Wang, S. (2022). Increased serum adenosine deaminase activity in patients with adult-onset Still's disease. BMC immunology, 23(1), 4.

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