Adenosine Deaminase

Microplate Assay Kit

User Manual

Catalog # CAK1290

(Version 1.1A)

Detection and Quantification of Adenosine Deaminase(AD) Content in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Adenosine Deaminase (AD, EC 3.5.4.4) is widely distributed in animal tissues. The highest enzyme activities are found in the mucosa of the small intestine, in the appendix and the spleen. The enzyme is present in the cytoplasmic cell fraction and also to some extent in the nucleus. The optimum pH is 7.0 to 7.4.

Adenosine DeaminaseMicroplate Assay Kitprovides a simple and sensitive method for monitoring Adenosine Deaminase activity in various samples. In this assay, Adenosine Deaminase catalyzes conversion of substrate into inosine and an intermediate, which reacts with a developer to form a colored product that absorbs maximally at 620 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Substrate	Powderx 1	4 °C
Dye ReagentI	Powderx 1	4 °C
Dye ReagentII	5 mlx 1	4 °C
Standard (1mmol/L)	1 mlx 1	4 °C
Positive Control	Powderx 1	-20 °C
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Note:

Substrate: add 1 mlReaction Buffer to dissolve before use, store at 4 °C for 1 month.

Dye ReagentI: add 5 ml distilled water to dissolve before use, store at 4 °C for 1 month.

Positive Control: add 1mlAssay Buffer before use, mix, store at -80°C for 1 month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm thesolution to room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	80 µl	80 µl	--	--	80 µl
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Substrate	10 µl	10 µl	--	--	10 µl
Standard	--	--	100 µl	--	--
Distilled water	--	--	--	100 µl	--
Dye ReagentI	50 µl	50 µl	50 µl	50 µl	50 µl
Dye Reagent II	50 µl	50 µl	50 µl	50 µl	50 µl
Mix,incubate at RT for 10 mins, record absorbance measured at 620 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Adenosine Deaminase activity is defined as the enzyme deaminate 1 μmol of adenosine to inosine per min at pH 7.4 at 25° C.

1. According to the protein concentration of sample

$$AD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$AD\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

$$AD\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$AD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 1 mmol/L = 1μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

$V_{Standard}$: the volume of the standard, 0.1 ml;

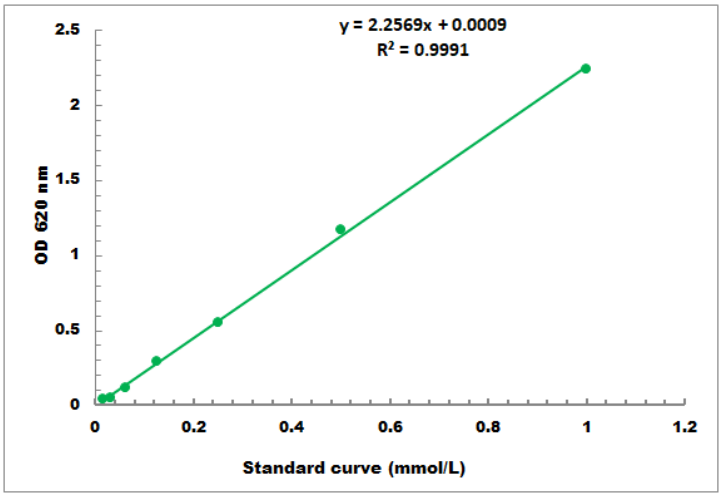
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

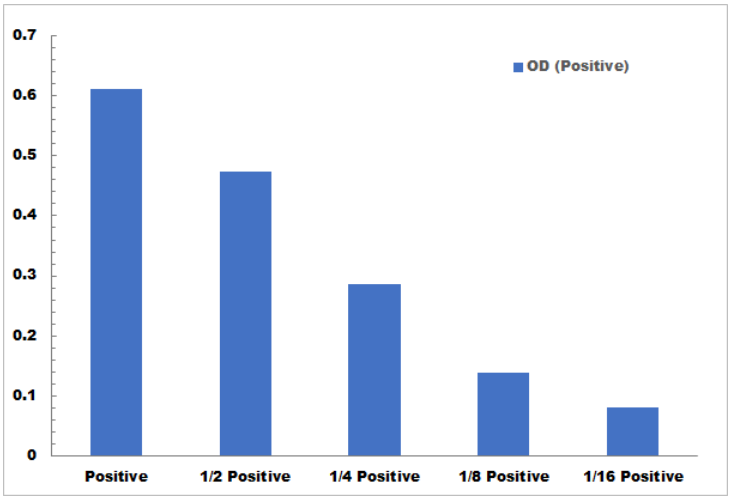
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01mmol/L -1mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES