



# **Adenosine Deaminase Microplate Assay Kit User Manual**

**Catalog # CAK1290**

(Version 1.1A)

Detection and Quantification of Adenosine Deaminase (ADA)

Activity in Tissue extracts, Cell lysate Samples.

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

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
IV. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

## I. INTRODUCTION

Adenosine Deaminase (ADA, 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 Deaminase Microplate Assay Kit provides 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 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent II Diluent	3 ml x 1	4 °C
Standard (1 mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

### Note:

**Substrate:** add 1 ml Reaction Buffer to dissolve before use. Store at -20 °C. Use within one month.

**Dye Reagent I:** add 7 ml distilled water to dissolve before use. Store at 4 °C. Use within one week.

**Dye Reagent II:** add 3 ml Dye Reagent II Diluent into Dye Reagent II, mix before use. Store at 4 °C. Use within one week.

**Positive Control:** add 1 ml Assay Buffer before use, mix. Aliquot & store at -80 °C. Use within one 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 ml Assay buffer for  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 20 minutes, 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 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly or dilute with Assay Buffer.

## V. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	80 $\mu$ l	80 $\mu$ l	--	--	80 $\mu$ l
Sample	10 $\mu$ l	--	--	--	--
Distilled water	--	10 $\mu$ l	--	--	--
Positive Control	--	--	--	--	10 $\mu$ l
Substrate	10 $\mu$ l	10 $\mu$ l	--	--	10 $\mu$ l
Standard	--	--	100 $\mu$ l	--	--
Distilled water	--	--	--	100 $\mu$ l	--
Dye Reagent I	70 $\mu$ l	70 $\mu$ l	70 $\mu$ l	70 $\mu$ l	70 $\mu$ l
Dye Reagent II	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l	30 $\mu$ l
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  $\mu\text{mol}$  of adenosine to inosine per min at pH 7.4 at 25° C.

### 1. According to the protein concentration of sample

$$\begin{aligned} \text{ADA (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (C_{\text{Protein}} \\ &\quad \times V_{\text{Sample}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned} \text{ADA (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad W / V_{\text{Assay}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

### 3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{ADA (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

### 4. According to the volume of sample

$$\begin{aligned} \text{ADA (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &\quad / T \\ &= (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the concentration of standard, 1 mmol/L = 1  $\mu\text{mol/ml}$ ;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

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

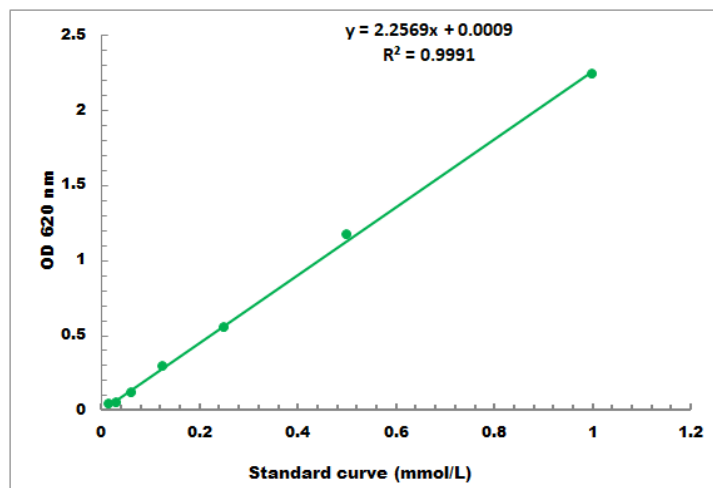
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

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

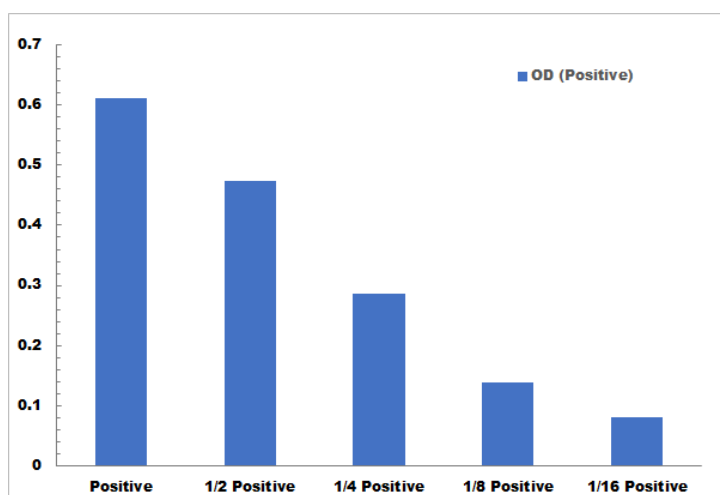
T: the reaction time, 10 minutes.

## VII. TYPICAL DATA

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



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



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

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES