Adenosine

Microplate Assay Kit

User Manual

Catalog # CAK1313

(Version 1.1A)

Detection and Quantification of Adenosine Content in Plasma, Tissue extracts, Cell lysate, Cell culture media, Urine, Other biological fluids Samples.

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

I. INTRODUCTION

Adenosine, a purine nucleoside, is present throughout the body. It plays an important role in energy transfer via the formation of ATP, ADP and AMP and in signal transduction via the formation of cAMP. Adenosine mediates its effects directly via adenosine receptors A1, A2A, A2B and A3. It regulates myocardial oxygen consumption and coronary blood flow, exerts anti-inflammatory effects throughout the body and also regulates the Renin-Angiotensin system. It also plays a role in tissue damage and repair, and cell death. Plasma adenosine levels are increased in patients with ischemic and non-ischemic heart failure.

Adenosine Microplate Assay Kit provides a simple and sensitive method for monitoring adenosine content in various samples. In this assay, adenosine deaminase catalyzes conversion of adenosine into inosine and ammonia, 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
Enzyme	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	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Dye Reagent I: add 7 ml distilled water to dissolve before use.

Dye Reagent II: add 3 ml Dye Reagent II Diluent into Dye Reagent II, mix before use.

Enzyme: add 1 ml Assay Buffer before use, mix, store at -80 °C for 1 month.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.3 ml distilled water, mix, the concentration will be 6 mmol/L.

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 5×10^6 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.

V. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	80 µl	80 µl	80 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Shake and mix, put it into the oven, 37 °C for 10 minutes.			
Dye Reagent I	70 µl	70 µl	70 µl
Dye Reagent II	30 µl	30 µl	30 µl
Shake and mix, put it into the oven, 37 °C for 30 minutes. Then 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

1. According to the protein concentration of sample

$$\text{Adenosine (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample})$$

$$= 6 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Adenosine (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Adenosine (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Adenosine (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 6 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 6 mmol/L = 6 μmol/ml;

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

W: the weight of sample, g;

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

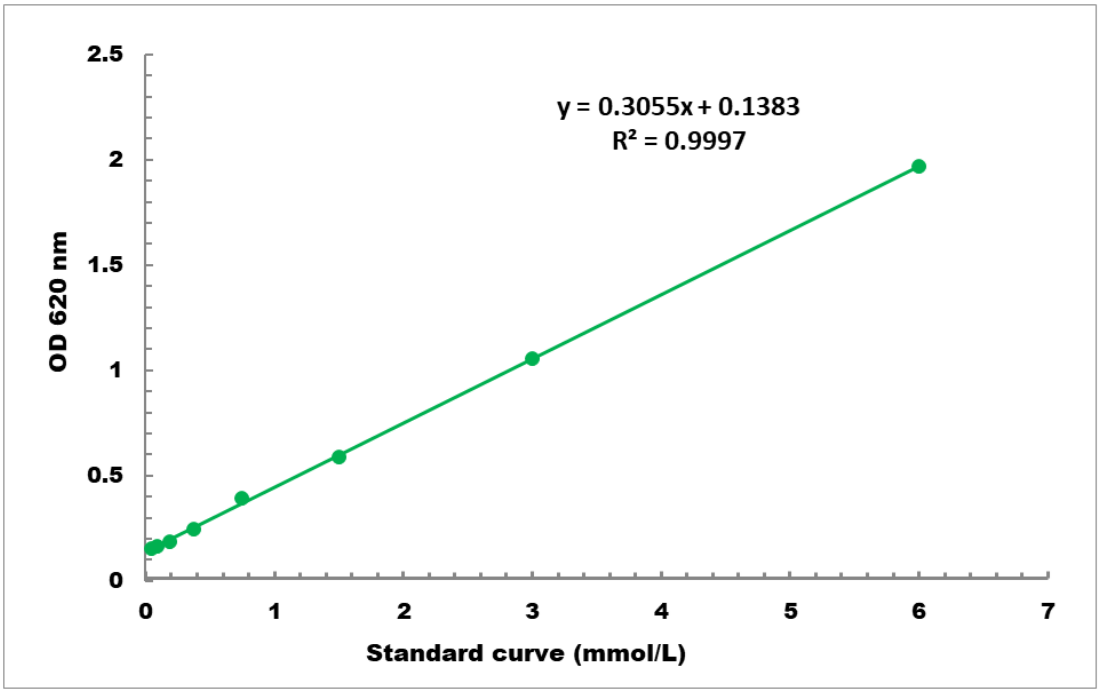
$V_{Standard}$: the volume of the standard, 0.01 ml;

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

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

VII. TYPICAL DATA

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



Detection Range: 0.06 mmol/L - 6 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES