Adenosine

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8001

(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.

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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 is measured using adenosine deaminase followed by a multi-step enzymatic approach resulting in the generation of an intermediate that reacts with the Adenosine Probe with the formation of a fluorescent product, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black 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
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20 °C. Use within one month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20 °C, protect from light and moisture. Use within one month.

Standard: add 1 ml distilled water to dissolve before use; then add 0.01 ml into 0.99 ml distilled water, mix, the concentration will be 150 µmol/L. Store at -20 °C. Use within one month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
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 with assay buffer.

Centrifuge urine sample at 4000 X g, 4°C for five minutes to remove any particulates.

V. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Probe	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 15 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.			

Note:

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

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

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

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

$$= 0.15 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.15 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 0.15 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

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

$$= 0.15 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 150 μmol/L = 0.15 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;

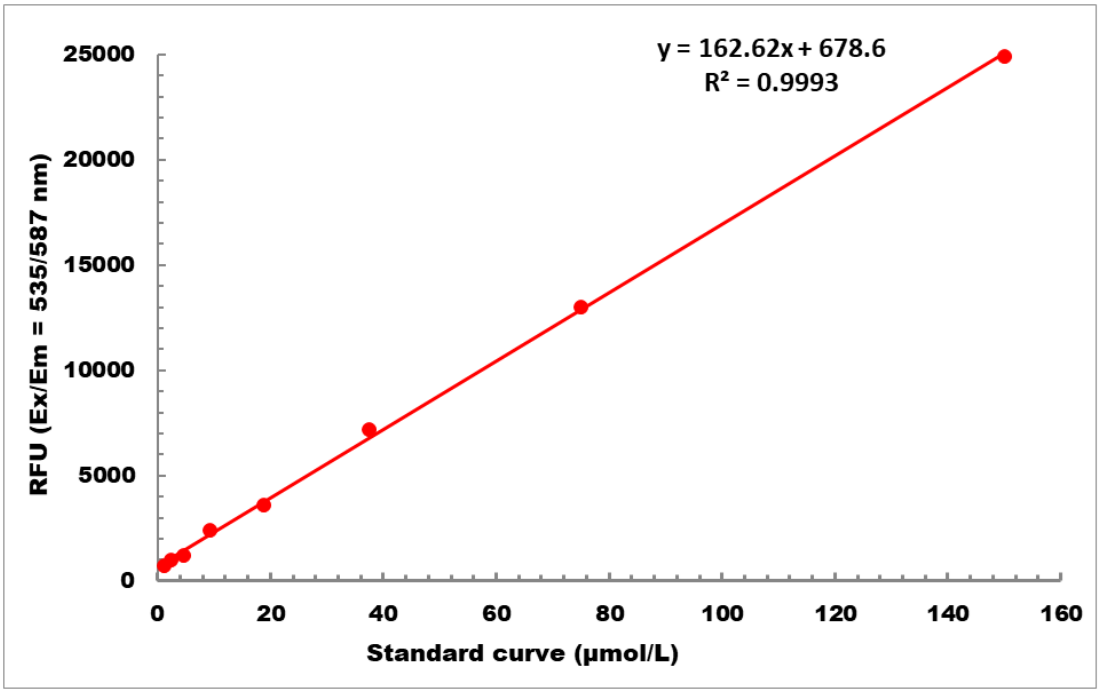
$V_{Standard}$: the volume of the standard, 0.01 ml;

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: 1 µmol/L - 150 µmol/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