Adenosine

FluorometricMicroplate Assay Kit

User Manual

Catalog # CAK8001

(Version 1.2A)

Detection and Quantification of AdenosineContent 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 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 mlx 4	4 °C
Reaction Buffer	10 mlx 1	4 °C
Enzyme	Powderx 1	-20 °C, keep in dark
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 mlx 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mldistilled waterto generate 15 mmol/L of standardstock solution,store at 4 °C for 1 monthafter reconstitution.Then dilute to150 µmol/Lstandard top solutionby adding 10 µl stock solution into 990µl distilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be150/75/37.5/18.75/9.37/4.68/2.34/1.17µmol/L.

Enzyme:Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use.Aliquot & store at -20 °C.Keep in dark and use within one month.

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

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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 liquid samples

Detect directlywith Assay Buffer.

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

VI. ASSAY PROCEDURE

Warm thesolution to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10µl	--	--
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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(RFU_{Sample}-RFU_{Blank})-Intercept}{Slope}\times n(\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard RFU and concentration

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(RFU_{Sample}-RFU_{Blank})}{(RFU_{Standard}-RFU_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

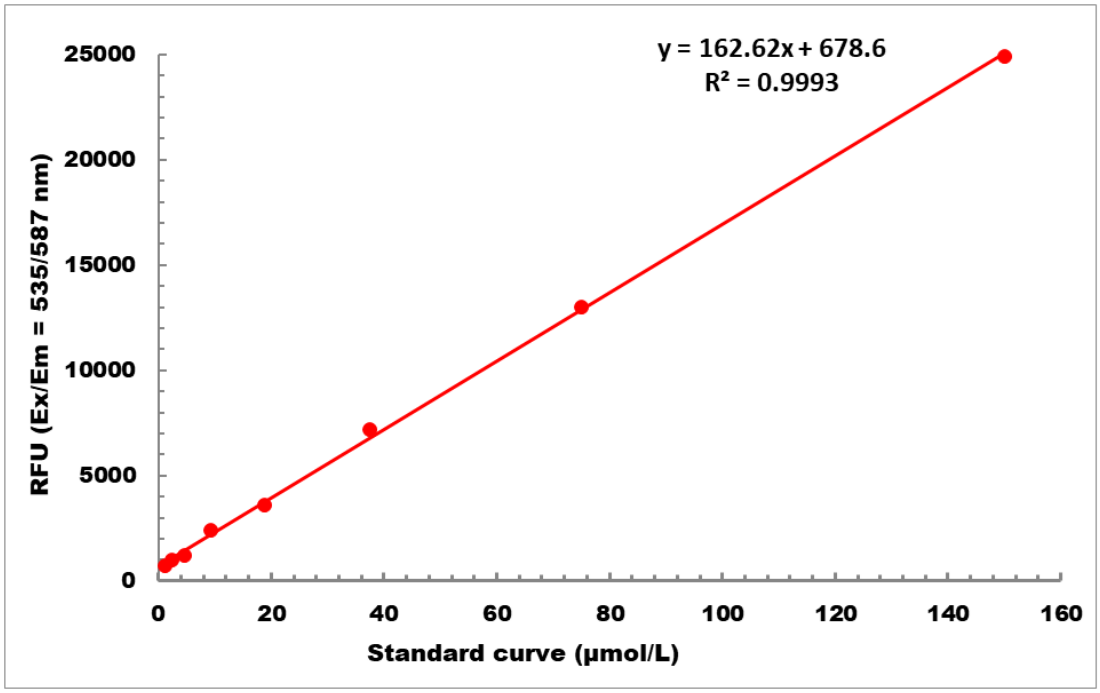
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

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

VIII. 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