Adenosine Fluorometric Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6891

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 .

1. Introduction

Adenosine, a purine nucleoside, is present throughout the body. It pl ays 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 A 1, A2A, A2B and A 3. It regulates myocardial oxygen consump tion 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 isc hemic 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).

2. 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	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 15 mmol/ L of standardstock solution,store at 4°C for 1 month after reconstitution. Then dilute to 150 μmol/ Lstandard top solution by adding 10 μl stock solution into 990μl distilled water. Perform 2 -fold serial dilutions of the top standard solution using distilled water to make the standard curve.The concentration of standard curve could be 150/75/37.5/18.75/9.37/4.68/2.34/ 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 ope ning then 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.

5. 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 10000 g 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 mL Assay Buffer on ice, centrifuged at 10000 g 4°C for 2 0minutes , 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.

6. Assay Procedure

Warm thesolution to room temperature before use . Add following reagents in to the 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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

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

Calculate the initial concentration according to sample preparation procedure .

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

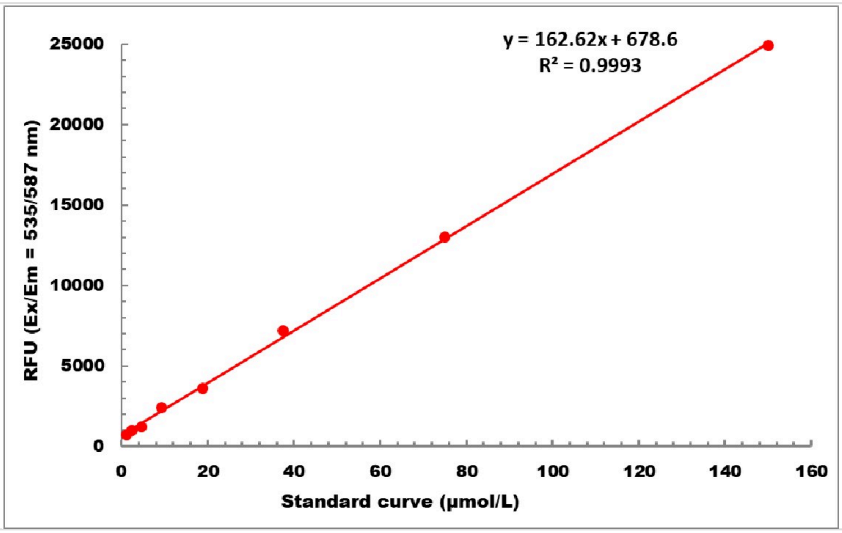
2.4According to the volume of sample

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

Item	Definition
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

8. 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