Product Manual

Adenosine Assay Kit

Catalog Number

MET-5090 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Adenosine is a purine nucleoside containing an adenine moiety attached to a ribose sugar molecule (ribofuranose) through a β-N9-glycosidic bond. Derivatives of adenosine perform an important role in energy transfer reactions (as adenosine triphosphate (ATP) and adenosine diphosphate (ADP)) as well as signal transduction (as cyclic adenosine monophosphate (cAMP)). Additionally, adenosine is a neuromodulator and is thought to promote sleep and suppress arousal. Adenosine also regulates blood flow to multiple organs through vasodilation. Adenosine is a byproduct of the enzymatic conversion of S-Adenosylhomocysteine (SAH) to homocysteine by Adenosylhomocystinease (AHCY).

Adenosine causes a temporary block of the atrioventricular (AV) node in the heart, while also relaxing smooth muscle found inside the artery walls. Dilation of the "normal" segments of arteries allows physicians to use adenosine to test for blockages in the coronary arteries, by exaggerating the difference between the normal and abnormal segments. In people suspected of having a supraventricular tachycardia (SVT), adenosine can be used to help identify the problem. Certain SVTs can be successfully stopped with adenosine. In addition, atrial tachycardia can sometimes be terminated with adenosine. Finally, adenosine is used in combination with thallous (thallium) chloride TI 201 or Tc99m myocardial perfusion scintigraphy (nuclear stress test for heart attack risk) in people who are unable to undergo sufficient stress testing with exercise.

Cell Biolabs' Adenosine Assay Kit is a simple fluorometric assay that measures the amount of total adenosine present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, adenosine standards, and unknown samples. Sample adenosine concentrations are determined by comparison with a known adenosine standard. The kit has a detection sensitivity limit of 1.56 µM adenosine.

Note: Each sample replicate requires 2 assays, one treated with adenosine deaminase (+ADA) and one without (-ADA). Adenosine is calculated from the difference in RFU readings from the 2 wells.

Assay Principle

Cell Biolabs' Adenosine Assay Kit measures total adenosine within biological samples. Adenosine is converted into inosine by adenosine deaminase (ADA). Then inosine is converted into hypoxanthine by purine nucleoside phosphorylase (PNP). Finally, hypoxanthine is converted to xanthine and hydrogen peroxide by xanthine oxidase (XO). The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of adenosine standard within the 96-well microtiter plate format. Samples and standards are incubated for 15 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

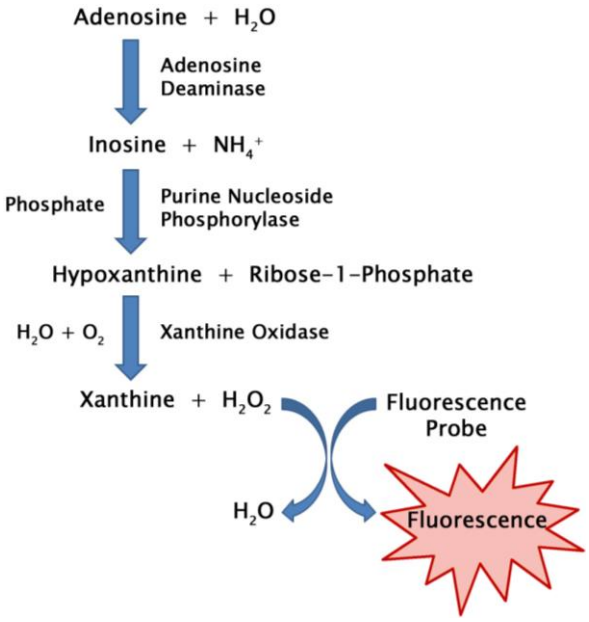

Figure 1. Adenosine Assay Principle.

Related Products

1. MET-5092: Inosine Assay Kit
2. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
3. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit

Kit Components

1. Adenosine Standard (Part No. 50901C): One 50 µL tube at 10 mM.
2. 10X Assay Buffer (Part No. 268002): One 25 mL bottle of 500 mM sodium phosphate pH 7.4.
3. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
4. HRP (Part No. 234402-T): One 10 µL tube of a 100 U/mL solution in glycerol.
5. Adenosine Deaminase (Part No. 50902C): One 10 µL tube at 1000 U/mL.

Note: One unit is defined as the amount of enzyme that will deaminate 1.0 µmole of adenosine to inosine per min. at pH 7.5 at 25°C.

6. Purine Nucleoside Phosphorylase (Part No. 50903D): One 500 µL tube at 18.9 U/mL.

Note: One unit is defined as the amount of enzyme that will cause the phosphorolysis of 1.0 µmole of inosine to hypoxanthine and ribose 1-phosphate per min at pH 7.4 at 25°C.

7. Xanthine Oxidase (Part No. 50904D): one 100 µL tube at 2.5 U/mL.

Note: One unit is defined as the amount of enzyme that will convert 1.0 µmole of xanthine to uric acid per min at pH 7.5 at 25°C. About 50% of the activity is obtained with hypoxanthine as substrate.

Materials Not Supplied

1. Phosphate Buffered Saline (PBS)
2. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate

Storage

Upon receipt, store the 10X Assay Buffer at room temperature and store the rest of the kit at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Note: After thawing any of the three enzymes for the first time, make smaller aliquots and store at -20°C.

Preparation of Reagents

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at room temperature.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, Adenosine Deaminase 1:500, Purine Nucleoside Phosphorylase 1:10, and Xanthine Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 2 µL of Adenosine Deaminase, 100 µL of Purine Nucleoside Phosphorylase, and 20 µL of Xanthine Oxidase to 866 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

Note: Prepare only enough for immediate use by scaling the above example proportionally.

- Control Mix: Prepare a Reaction Mix (without adenosine deaminase) by diluting the Fluorometric Probe 1:100, HRP 1:500, Purine Nucleoside Phosphorylase 1:10, and Xanthine Oxidase 1:50 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 100 µL of Purine Nucleoside Phosphorylase, and 20 µL of Xanthine Oxidase to 868 µL of 1X Assay Buffer for a total of 1 mL. This Control Mix volume is enough for 20 assays. The Control Mix is stable for 1 day at 4ºC.

Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell culture supernatants: Cell culture media containing adenosine, inosine, xanthine, and hypoxanthine should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary in PBS.

Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant may be assayed directly or diluted as necessary in PBS.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in PBS.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it*

is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).

- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Adenosine standards according to Table 1 below.

Standard Tubes	10 mM Adenosine Solution (µL)	PBS (µL)	Adenosine (µM)
1	5	495	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.56
8	0	250	0

Table 1. Preparation of Adenosine Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

Note: Each sample replicate requires two paired wells, one to be treated with Adenosine Deaminase (+ADA) and one without the enzyme (-ADA) to measure endogenous background.

2. Add 50 µL of each standard into wells of a black microtiter plate suitable for a fluorescence plate reader.
3. Add 50 µL of each unknown sample to each of two separate wells.
4. Add 50 µL of Reaction Mix to all standard wells and one half of the paired sample wells.
5. Add 50 µL of Control Mix to the remaining paired sample wells.
6. Mix the well contents thoroughly and incubate for 15 minutes at room temperature protected from light.

Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Example of Results

The following figure demonstrates typical Adenosine Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

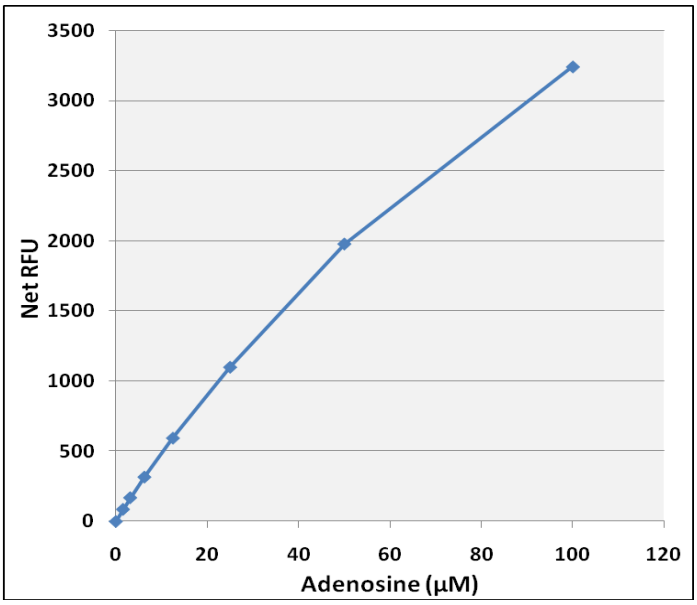

Figure 2: Adenosine Standard Curve.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Adenosine Deaminase (-ADA) from the sample well values containing Adenosine Deaminase (+ADA) to obtain the difference. The fluorescence difference is due to the Adenosine Deaminase activity.

$$\textbf{Net RFU} = (\textbf{RFU}_{\textbf{+ADA}}) - (\textbf{RFU}_{\textbf{-ADA}})$$

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of Adenosine present in the sample. Only use values within the range of the standard curve.

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Recent Product Citations

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