Adenosine Assay kit

ARG83372 Adenosine Assay kit is an assay kit for Adenosine in Serum, plasma, urine, cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83372

Package: 100 assay

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INTRODUCTION

Adenosine is a ribonucleoside composed of a molecule of adenine attached to a ribofuranose moiety via a beta-N(9)-glycosidic bond. It has a role as an anti-arrhythmia drug, a vasodilator agent, an analgesic, a human metabolite and a fundamental metabolite. It is a purines D-ribonucleoside and a member of adenosines. It is functionally related to an adenine.

PRINCIPLE OF THE ASSAY

ARG83372 Adenosine Assay Kit measures Adenosine content 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). Samples are compared to a known concentration of Adenosine standard within the 96-well microtiter plate format. The intensity of the color is measured at a wavelength of 540/590 nm. The concentration of Adenosine in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 20X Assay Buffer at RT.

Store other component at ≤ -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (10 mM)	50 µL	-20°C
10X Assay Buffer	25 ml	RT
Adenosine Deaminase	10 µL	-20°C
Purine Nucleoside	500 µL	-20°C
Xanthine Oxidase	100 µL	-20°C
Probe	50 µL	-20°C
HRP-Streptavidin Solution	10 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540/590 nm
- Flat bottomed 96-well black microplate and tube.
- 1X PBS and deionized water
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Store 10X Assay Buffer at RT. Store other component at ≤ -20°C. Use the kit before expiration date. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with Deionized Water to yield 1X Assay Buffer. Store at RT.
- **Reaction Mix:** Dilute the Probe at 1:100, HRP at 1:500, Adenosine Deaminase at 1:500, Purine Nucleoside Phosphorylase at 1:10, and Xanthine Oxidase at 1:50 in 1X Assay Buffer. For 20 assays, add 10 µL 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. Store the Reaction Mix at 4°C for 1 day.
- **Control Mix:** Dilute the Probe at 1:100, HRP at 1:500, Purine Nucleoside Phosphorylase at 1:10, and Xanthine Oxidase at 1:50 in 1X Assay Buffer. For 20 assays, add 10 µL 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. Store the Reaction Mix at 4°C for 1 day.

- **Standards**: Add 5 µl of 10.0 mM stock standard into 495 µl PBS to generate a standard with 100 µM of Tyrosine. Dilute the standards with PBS serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Adenosine (µM)	Volume of PBS (µL)	Volume of Adenosine(µL)
S1	100	495	5 (10 mM stock)
S2	50	250	250(S1)
S3	25	250	250(S2)
S4	12.5	250	250(S3)
S5	6.25	250	250(S4)
S6	3.13	250	250(S5)
S7	1.56	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

Each samples should be assayed in at least duplicates, one to be treated with Adenosine Deaminase and one without the enzyme to measure endogenous background.

1. Add **50 µl** of **standard** and **sample** to each wells.
2. Add **50 µL** of **Reaction Mix** to standard and half of sample wells.
3. Add **50 µL** of **Control Mix** to other half of sample wells.
4. Mix well and Incubate for 15 min at RT.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
5. Read O.D. with a microplate reader at **590 nm** immediately.

CALCULATION OF RESULTS

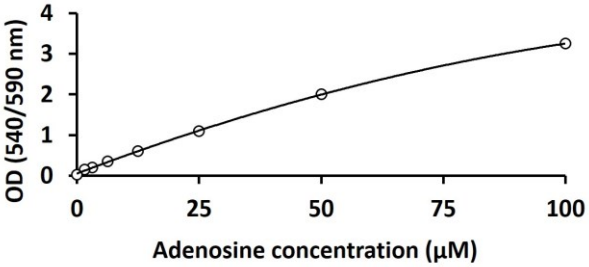
1. Calculate the average absorbance values for each set of standards sample and control.
2. Subtract the sample well values without Adenosine Deaminase from the sample well values containing Adenosine Deaminase to obtain the difference.

$$\Delta OD = (OD_{+ADA}) - (OD_{-ADA})$$

3. Compare the ΔOD of each sample to the standard curve to determine and extrapolate the quantity of Adenosine present in the sample.

EXAMPLE OF TYPICAL STANDARD CURVE

The following table shows the OD readings of a run of this assay kit with serial diluted standards



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Adenosine ranged from 1.56-100 µM. The mean MDD was 0.85 µM