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

For research use only. Not for use in diagnostic procedures.

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MANUFACTURED BY:

arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

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). 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store 10X 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 fluorometric measurement
- 96-well fluorescence 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.
- The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.
- After thawing any of the three enzymes for the first time, make smaller aliquots and store at -20°C.

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

Cell Lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. 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.

Notes:

1. 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.

2. 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

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

REAGENT PREPARATION

- 1X Assay Buffer: Dilute the 10X Assay Buffer with Deionized Water to yield 1X Assay Buffer. Stir or vortex to homogeneity. 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.

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

- **Standards**: Add 5 µl of 10.0 mM stock standard into 495 µl PBS to generate a standard with 100 µM of Adenosine. 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

- **Sample:** 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 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 protected from light.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
5. 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.

CALCULATION OF RESULTS

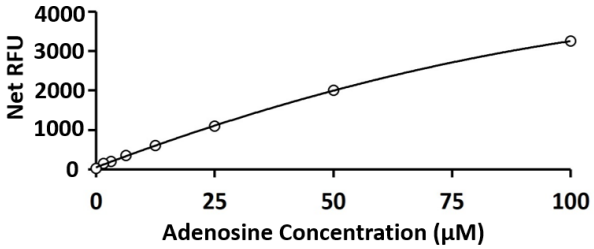
1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Subtract the sample well values without Adenosine Deaminase from the sample well values containing Adenosine Deaminase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

$$\text{Net RFU} = (RFU_{+ADA}) - (RFU_{-ADA})$$

4. 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.

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