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Adenosine Assay Kit (Fluorometric)

4/15

(Catalog # K327-100; 100 reactions; Store at -20°C)

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. In BioVision's Adenosine 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. The fluorescent product is measured at Ex/Em = 535/587 nm. Detection range: 2-80 pmol.

Adenosine $\xrightarrow{\text{Detector, Convertor \& Developer}}$ Intermediate + Adenosine Probe $\longrightarrow$ Fluorescent Product (Ex/Em = 535/587 nm)

II. Application:

- Measurement of Adenosine in plasma and urine

III. Sample Type:

- Plasma and urine

IV. Kit Contents:

Components	K327-100	Cap Code	Part Number
Adenosine Assay Buffer	25 ml	WM	K327-100-1
Urine Clarifier	1 vial	Brown	K327-100-2
Adenosine Detector	1 vial	Green	K327-100-3
Adenosine Convertor	1 vial	Clear	K327-100-4
Adenosine Developer	1 vial	Orange	K327-100-5
Adenosine Standard (10 mM)	100 µl	Yellow	K327-100-6
Adenosine Probe (DMSO)	200 µl	Red	K327-100-7

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer capable of fluorescence read out
- Immobilized Catalase Beads (Cat. # 7931) for urine samples.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Adenosine Assay Buffer**: Bring to room temperature before use. Store at -20°C.
- **Urine Clarifier, Adenosine Detector, and Adenosine Developer:** Reconstitute each vial with 220 µl Adenosine Assay Buffer. Pipette gently to dissolve. Aliquot and store at -20°C. Use within two months after reconstitution. Keep on ice while in use. Use within two months after reconstitution.
- **Adenosine Convertor:** Reconstitute with 440 µl Adenosine Assay Buffer. Pipette gently to dissolve. Aliquot and store at -20°C. Keep on ice while in use. Use within two months after reconstitution.

VII. Adenosine Assay Protocol:

1. **Sample Preparation:** Add 5-20 µl undiluted plasma or 1-5 µl of pre-treated urine (2x diluted during the pretreatment method or further diluted 2x with Adenosine Assay Buffer to give 4x diluted urine) into desired well(s) in a 96-well plate and adjust the volume to 50 µl with Adenosine Assay Buffer. For each sample, prepare two wells each, one as sample background and the other as sample test reaction.

   Notes:

   a) Inosine**,** xanthine and hypoxanthine present in the sample(s) will contribute to the background.

   b) For unknown samples, we suggest doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range. Dilute samples if necessary.

   c) We recommend using fresh plasma or flash frozen plasma stored at -80°C.

   d) Centrifuge urine sample at 1000 X g, 4°C for five min. to remove any particulates. To pre-treat urine, add 2 µl each of Adenosine Convertor and Urine Clarifier and 10 µl of a 50 % suspension of Catalase Beads (Cat. # 7931, not provided) to 50 µl urine sample. Adjust the volume to 100 µl with Adenosine Assay Buffer and incubate at room temperature for 15 min. Centrifuge this pre-treated urine at 1000 x g for one min. and transfer the supernatant to a fresh tube. Store the pretreated urine sample on ice for immediate use. Sample can be stored at -80°C for future analysis. If using more than one urine sample, the pretreatment can be carried out in a 96-well plate.

2. **Standard Curve Preparation:** Dilute Adenosine Standard to 1 mM by adding 10 µl of 10 mM Adenosine Standard to 90 µl Adenosine Assay Buffer. Further dilute the Adenosine Standard to 10 µM by adding 10 µl of 1 mM Adenosine to 990 µl Adenosine Assay Buffer.

Add 0, 2, 4, 6, and 8 µl of diluted 10 µM Adenosine Standard into a series of wells in 96-well plate to generate 0, 20, 40, 60, and 80 pmol/well Adenosine Standard. Adjust the volume to 50 µl/well with Adenosine Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For Standards and sample test reactions, prepare 50 µl Reaction Mix and for sample background, prepare 50 µl Background Control Mix containing:

	Reaction Mix	Background Control Mix
Adenosine Assay Buffer	43 µl	45 µl
Adenosine Detector	2 µl	–
Adenosine Convertor	2 µl	2 µl
Adenosine Developer	2 µl	2 µl
Adenosine Probe*	1 µl	1 µl

Mix well. Add 50 µl Reaction Mix to each well containing Standard and sample test reaction and 50 µl of the Background Control Mix to each well containing sample Background. Mix well.

Note: * For testing urine samples, use 2 µl Adenosine Probe.

4. **Measurement:** Incubate at room temperature for 15 min., protected from light. Measure fluorescence (Ex/Em = 535/587 nm) in a plate reader.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Adenosine Standard Curve. For samples, correct sample background by subtracting the value of each sample background from respective Sample reading. Apply the corrected sample reading to the Adenosine Standard Curve to get B pmol of Adenosine in the sample well.

Sample Adenosine concentration (C) = B/V X D pmol/µl or µM

Where: **B** is amount of Adenosine in the sample well from Standard Curve (pmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

Adenosine in urine is expressed as µmol adenosine/mmol creatinine

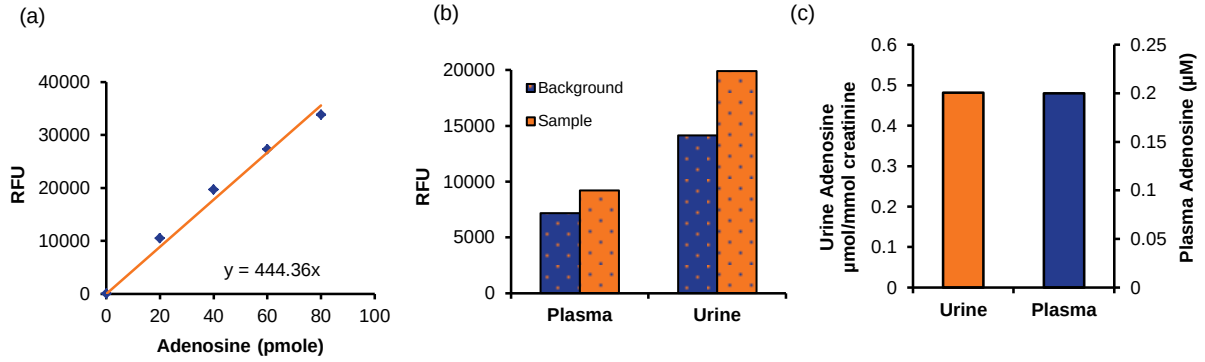

Figure: Adenosine Standard Curve (a). Measurement of Adenosine in pooled human plasma (20 µl) and human urine (4 µl of pre-treated urine, 2 times diluted during the pretreatment method) (b). Adenosine amount in human plasma and human urine (c). Assays were performed following the kit protocol.

VIII. Related Products:

Xanthine Oxidase Colorimetric/Fluorometric Assay Kit (K710)
Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (K685)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ATP Colorimetric/Fluorometric Assay Kit (354)
AHCY Inhibitor Screening Kit (K326)
Bioluminescence Cytotoxicity Assay Kit (K312)
Adenosine Deaminase Activity Assay (Fluorometric) (K328)
Inosine Fluorometric Assay Kit (K712)
Uric Acid Colorimetric/Fluorometric Assay Kit (K608)
ADP Colorimetric Assay Kit II (K356)
AHCY Activity Assay Kit (K807)
cAMP Direct Immunoassay Kit (Colorimetric) (K371)
Immobilized Catalase Beads (7931)
Creatinine Colorimetric/Fluorometric Assay Kit (K625)

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