

Adenosine Fluorometric Assay

(Catalog: TBS2083, 100 Assays, Store at -20°C)

DESCRIPTION

Adenosine is a vital endogenous purine nucleoside that plays a key role in cellular energy metabolism and extracellular signaling. Quantifying adenosine levels is essential for investigating purinergic signaling pathways linked to ATP, ADP, cAMP, and inflammatory responses. Furthermore, disruptions in adenosine metabolism and signaling are closely associated with hypoxia, ischemia, inflammation, cardiovascular diseases, neurological disorders, cancer, and immune regulation.

The **Tribioscience Adenosine Fluorometric Assay** provides a flexible, accurate, simple, and time-saving method for the quantitative measurement of adenosine. The assay relies on a coupled enzyme reaction that yields an intermediate product. This intermediate then reacts with a specialized probe to generate a fluorescence shift measured at Ex/Em = 530/590 nm, where the resulting fluorescence intensity directly correlates with the concentration of adenosine in the sample.

SYNONYMS

Ade-Rib, Adenine riboside, D-adenosine, Adenogesis

APPLICATIONS

Adenosine in cells and various biological samples.

KIT CONTENTS (100 TESTS)

Part Name	Part Size
Assay Buffer	20 mL
Enzyme Mix	50 µL
Assay Developer	35 µL
Probe	240 µL
Adenosine Standard (5 mM)	50 µL

STORAGE AND HANDLING

Store the kit at -20°C. The shelf life is 12 months.

ASSAY PROTOCOL

Except Enzyme, warm all the components to room temperature before use. Briefly centrifuge all small vials prior to opening.

1. Sample Preparation:

1.1 Tissue (1-10 mg) or cells (1×10^6) can be lysed in 100 µL of Assay Buffer. Centrifuge ice cold at $15,000 \times g$ for 2 minutes to pellet insoluble materials and collect supernatant. For more accurate assay, the sample should be quickly frozen using liquid N₂ or dry ice if it is to be assayed later.

1.2 Centrifuge urine or plasma sample at 1000 g at 4°C for 5 minutes to remove any particulates.

2. Standard Curve Preparations:

2.1 Label 1.5mL tubes 1-8 for a standard curve preparation displayed as Table 1. Add 288µL of 1x Assay Buffer to Std 1 and 150µL to Std 2-8.

2.2 Add 12 µL of 5 mM Adenosine Stock solution to Std1 and fully mix,

2.3 Carry out a 2x serial dilution sequentially for Std 2-7 by transferring 150µL of Std1 to Std2 and afterwards. Leave Std 8

as the 0 standard (the assay buffer alone as blank). The standards concentrations are 200, 100, 50, 25, 12.5, 6.25, 3.13, and 0 µM for Std 1-8.

Table1: Assay Standard Preparation

Tubes	Adenosine (µL)	Buffer	Adenosine (µM)
1	12 µL of 5 mM stock	288 µL	200
2	150 µL of Tube#1	150 µL	100
3	150 µL of Tube#2	150 µL	50
4	150 µL of Tube#3	150 µL	25
5	150 µL of Tube#4	150 µL	12.5
6	150 µL of Tube#5	150 µL	6.25
7	150 µL of Tube#6	150 µL	3.13
8	0	150 µL	0

3. Reaction Mix:

Prepare 5000 µL of reaction mix for 100 wells test as Table 2:

Table 2: Reaction Mix Preparation

Reaction Components	Volume
Assay Buffer	4728 µL
Enzyme mix	45 µL
Assay developer	27 µL
Probe	200 µL
Final Volume (µL)	5000 µL

4. Load Sample and Reaction Mix:

4.1 Add 50 µL/well standards or samples or control to 96-well plate (Note: use black plate for fluorometric assay).

4.2 Add 50 µL of the Reaction Mix to each well containing the Standards, Control and test samples. Tap the plate lightly to mix. Protect the plate from light during the incubation.

5. Measurement:

Measure the absorbance value at fluorescence at Ex/Em = 530/590 nm in fluorometric mode for 30-60 min at 37°C (Note: the incubation time is dependent on the adenosine in the samples).

6. Calculation:

Correct background by subtracting the value of the 0 standard (blank) from all readings to get ΔRFU. Plot the standard curve. Apply the corrected sample value to the standard curve to get adenosine µM based on the standard curve (e.g. The formula on standard curve: $Y=aX+C$).

$$\text{Sample Adenosine Concentration (µM)} = DF * (Y-C)/a$$

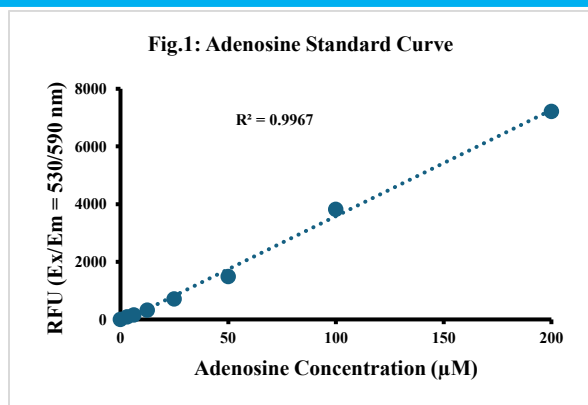
Where: Y as sample ΔRFU, C: Y-intercept, a: the slope of the line). DF: dilution factor

7. Typical Standard Curve for Fluorometric Assays:

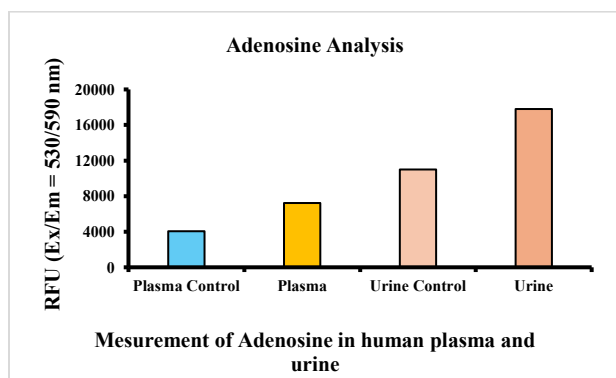
The typical standard curve was shown in Fig. 1. The Typical standard curve was only used as a reference for assay. It cannot be used for real standard curve for assay.

Adenosine Fluorometric Assay

(Catalog: TBS2083, 100 Assays, Store at -20°C)



The Adenosine curve in different samples was shown in Fig. 2



RELATED PRODUCTS

CDK4 Fluorometric Assay (TBS2127)
 ETNK Fluorometric Assay (TBS2209)
 EGFR Activity Assay (TBS2048)
 LDH Cytotoxicity Assay (TBS2002)
 ATP Colorimetric/Fluorometric Assay (TBS2010)
 ADP Colorimetric/Fluorometric Assay (TBS2020)
 XTT Cell Viability Assay (TBS2021)
 Cell Count Kit -8 (TBS2022)
 Thiol Fluorometric Assay (TBS2026)
 GSH Assay (TBS2028)
 NAD/NADH Colorimetric Assay (TBS2029)
 Caspase-3 Colorimetric Assay (TBS2030)
 Pyruvate Colorimetric Assay (TBS2023C)
 Glycerol Colorimetric Assay (TBS2204C)
 Triglyceride Colorimetric Assay (TBS2205C)
 NNMT Inhibitor Screening Assay (TBS2097)
 NNMT Activity Fluorometric Assay (TBS2098)
 G6PDH Activity Colorimetric Assay (TBS2102)
 Cytochrome c Reductase Activity Assay (TBS2116)
 Mitochondria Complex 1 Activity Assay (TBS2017)
 Mitochondria Oxidase Activity (TBS2105)
 Mitochondrial Membrane Potential Assay (TBS2049)
 Cytochrome C Oxidase (Complex IV) Activity Assay (TBS2115)

This product is for research only.