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Product Information

Adenosine Quantification Assay Kit

Catalog Number **MAK433**
Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

Adenosine is a purine nucleoside base, which is the precursor of adenosine triphosphate (ATP) and is utilized thoroughly throughout the entire body in general metabolism.[1] Extracellular adenosine acts as a local modulator with a generally cytoprotective function in the body.[2] Its effects on tissue protection and repair fall into four categories: increasing the ratio of oxygen supply to demand; protecting against ischaemic damage by cell conditioning; triggering anti-inflammatory responses; and the promotion of angiogenesis.[3]

The Adenosine Quantification Assay Kit provides a simple and quick fluorometric method for the detection of adenosine. The kit is based on a multi-step enzymatic approach, resulting in the formation of fluorescent product that is measured at λ_{Ex} = 550 nm/λ_{Em} = 585.The kit has a linear range of 0.5 to 20 μM.

The kit is suitable for the quantification of adenosine in plasma.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

Assay Buffer Catalog Number MAK433A	50 mL
Adenosine Deaminase Catalog Number MAK433B	200 μL
Enzyme Mix Catalog Number MAK433C	2×1 mL
Probe Catalog Number MAK433D	100 μL
Adenosine Standard Catalog Number MAK433E	50 μL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Multiwell plate reader (equipped with 535 nm and 585 nm filters)
- Flat-bottom black 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C. Assay Buffer should be stored at 2–8 °C upon thawing. The unopened kit is stable for 2 years as supplied.

Preparation Instructions

Assay Buffer
Ready-to-use. Upon thawing, store at 2–8 °C.

Adenosine Deaminase
Ready-to-use solution. Store at –20 °C.

Enzyme Mix
Provided as a 2.5× concentrate. Avoid freeze/thaw cycles. It is recommended to prepare aliquots, and store the aliquots at –20 °C.

Probe
Provided as a 50× concentrate. Avoid freeze/thaw cycles. It is recommended to prepare aliquots, and store the aliquots at –20 °C, protected from light.

Adenosine Standard
Provided as a 10 mM solution. Store at –20 °C.

Procedure

Notes

- The assay is formatted for a 96-well microplate.
- Black 96-well microplates suitable for fluorescence plate reader should be used.
- All standards and samples should be run in duplicate.
- Equilibrate all reagents to room temperature before use.
- A fresh set of standards should be prepared for every use.
- Briefly centrifuge vials before opening.
- All assays (samples, standards and blank) require 50 µL for each reaction (well). Samples and standards should be diluted with Assay Buffer.
- Collect blood using either EDTA or heparin treated tubes.
- For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.
- **For each sample, an additional reaction without Adenosine Deaminase should be prepared and measured simultaneously as a sample blank.** Adenosine concentration is calculated from the difference between readings of sample and its appropriate blank.
- **For convenience, an Excel-based calculation sheet is available on the MAK433 Product Detail Page.** Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Standard Curve Preparation

1. Prepare two adenosine standard working solutions:
   a. 100 µM adenosine working solution - Dilute the Adenosine Standard (10 mM) 100-fold with Assay Buffer: 5 µL of Adenosine Standard with 495 µL of Assay Buffer.
   b. 10 µM adenosine working solution - Dilute the 100 µM adenosine working solution 10-fold with Assay Buffer: 20 µL of Adenosine Standard with 180 µL of Assay Buffer.
2. Add 0, 5, 12.5 and 25 µL of 10 µM adenosine working solution, and 5, 10, 15, and 20 µL of 100 µM adenosine working solution into wells of a 96-well plate, generating 0 (blank), 0.5, 1.25, 2.5, 5, 10, 15 and 20 µM/well standards. Bring the volume to 100 µL with Assay Buffer (see Table 1).

Table 1.
Preparation of adenosine standards

Adenosine working solution	Adenosine volume (µL)	Assay Buffer volume (µL)	Final conc. (µM)	well
100 µM	20	80	20	A1
	15	85	15	B1
	10	90	10	C1
	5	95	5	D1
10 µM	25	75	2.5	E1
	12.5	87.5	1.25	F1
	5	95	0.5	G1
N/A	0	100	0 (blank)	H1

3. Transfer 50 µL from each well to its adjacent well, to generate duplicates with a final volume of 50 µL per well: From well A1 transfer 50 µL to well A2, from well B1 transfer 50 µL to well B2, etc.

Sample Preparation:

1. Prepare deproteinated plasma by passing fresh plasma through a 10 kDa filter such as Corning Spin-X UF concentrator. Collect the flow-through. Note: To avoid adenosine loss, the plasma should be deproteinated immediately upon collection.
2. Dilute the deproteinated plasma with Assay Buffer 2- to 10-fold.

Reaction mix preparation:

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of reaction mix according to Table 2. Multiply the volumes in Table 2 according to the number of wells in the assay.
Note: For each sample, a sample blank should be prepared. This blank is devoid of adenosine deaminase.

Table 2.
Preparation of reaction mix

Reagent	Catalog Number	Standards & Samples	Sample Blank
Assay Buffer	MAK433A	28 µL	29µL
Adenosine Deaminase	MAK433B	1 µL	-
Enzyme Mix	MAK433C	20 µL	20 µL
Probe	MAK433D	1 µL	1 µL

Assay protocol:

1. Add 50 µL of sample to each sample well.
2. Add 50 µL of sample to each sample blank well.
3. Add 50 µL of reaction mix to each standard and sample well. Do **not** add reaction mix containing Adenosine Deaminase to each sample blank well.
4. Add 50 µL of reaction mix devoid of Adenosine Deaminase to each sample blank well.
5. Incubate the reaction for 15 minutes at room temperature, protected from light.
6. Measure the fluorescence with an excitation of 550-570 nm and emission of 585-595 nm.

Results

Notes

- **An Excel-based calculation sheet is available at the MAK433 Product Detail Page.** Use this sheet to calculate the test results.
- If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

1. Average the Relative Fluorescence Units (RFU) for each standard, sample and sample blank.
2. Subtract the averaged zero standard blank value (no adenosine) from all standards.
3. Subtract the averaged sample blank RFU value of each sample from its respective averaged sample value:

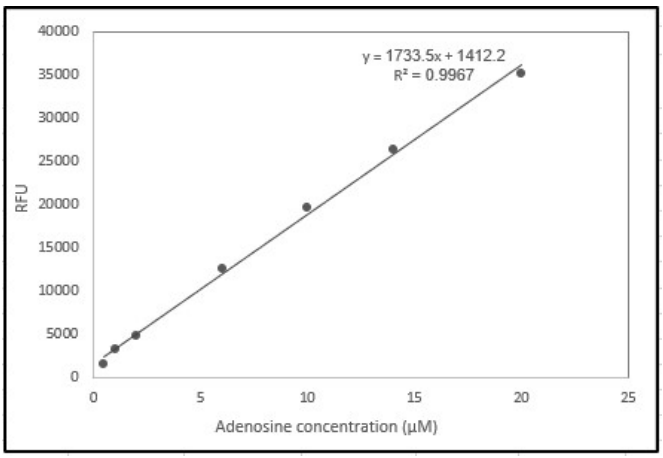
$$\text{Corrected RFU} = \text{RFU}_{(\text{sample})} - \text{RFU}_{(\text{sample blank})}$$

4. Plot the average RFU measured for each standard against the standard concentration and determine the linear regression equation.
5. Use the linear regression equation to calculate the adenosine concentration of the sample:

$$\mu\text{M adenosine} = \text{DF} \times \frac{(\text{Corrected RFU} - \text{intercept})}{\text{Slope}}$$

Where:
DF = Sample dilution factor (if sample is not diluted, the DF value is 1)
Corrected RFU = Value of subtracted RFU (see Step 3 above)

Figure 1.
Typical adenosine standard curve using MAK433 kit and protocol.



References

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2. Fredholm, B.B., et al., Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.,* **53**, 527–552 (2001).
3. Jacobson, K.A. and Gao, Z.-G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discov.,* **5(3)**, 247-264 (2006).

HM, NA, YM, VC 12/20-1