（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

**Catalog No：E-BC-K833-M**

**Specification：96T(40 samples)**

**Measuring instrument：Microplate reader(330-350 nm)**

**Detection range：0.027-1.000 mmol/L**

# **Elabscience®** Adenosine Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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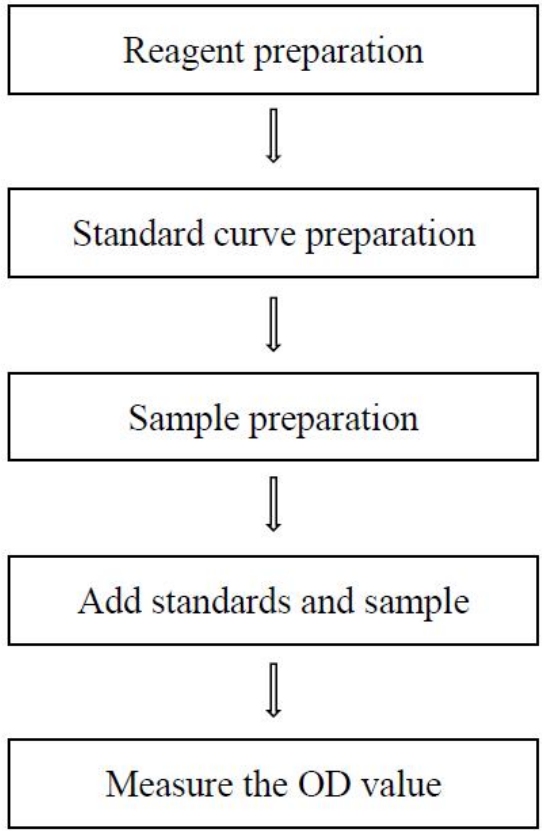
**Assay summary**



## Intended use

This kit can be used to measure adenosine content in serum (plasma), animal (plant) tissue and cell samples.

## Detection principle

Adenosine is an endogenous nucleoside distributed throughout human cells. It can directly enter the myocardium and be phosphorylated to produce adenosine, which is involved in myocardial energy metabolism, and also participates in the dilation of coronary vessels and increase blood flow. Adenosine has physiological effects on the cardiovascular system and many other systems and tissues of the body. It also promotes the growth of human hair follicles and the proliferation of dermal papilla cells in vitro.

The detection principle of this kit is that the substances produced by the enzyme-catalyzed adenosine reaction consume NADH, the OD value of the system decreases at 340 nm, and the content of adenosine can be calculated by calculating the decline rate at 340 nm.

## Kit components & storage

| Item | Component | Size (96 T) | Storage |
|---|---|---|---|
| Reagent 1 | Buffer Solution | 25 mL × 1 vial | -20℃, 12 months |
| Reagent 2 | Enzyme Reagent | 1 mL × 2 vials | -20℃, 12 months, shading light |
| Reagent 3 | Substrate | 1.5 mL × 1 vial | -20℃, 12 months, shading light |
| Reagent 4 | Accelerant | Powder × 2 vials | -20℃, 12 months, shading light |
| Reagent 5 | Catalyst | 1.5 mL × 2 vials | -20℃, 12 months, shading light |
| Reagent 6 | 10 mmol/L Standard | 0.5 mL × 1 vial | -20℃, 12 months, shading light |
| | UV Microplate | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

**Instruments:**

Microplate reader (330-350 nm, optimum wavelength: 340 nm),
Ultrasonic apparatus

**Consumptive material:**

10kDa MWCO Spin Filter

## Reagent preparation

① Equilibrate all the reagents to 25°C before use

② The preparation of accelerant working solution:

Dissolve one vial of accelerant with 0.75 mL of buffer solution, mix well to dissolve. Keep it on ice for detection and store at -20°C for 5 days protected from light.

③ The preparation of reaction working solution:

Before testing, please prepare sufficient reaction working solution. For example, prepare 162 μL of reaction working solution (mix well 120 μL of buffer solution, 12 μL of substrate, 6 μL of accelerant and 24 μL of catalyst). The reaction working solution should be prepared on spot, keep it on ice for detection and use it up the same day.

④ The preparation of 1 mmol/L standard solution:

Before testing, please prepare sufficient 1 mmol/L standard solution. For example, prepare 1000 μL of 1 mmol/L standard solution (mix well 100 μL of 10 mmol/L standard and 900 μL of buffer solution). Keep it on ice for detection and use it up within 2 days.

⑤ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.5, 0.6, 0.8, 0.9, 1 mmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mmol/L)** | **0** | **0.2** | **0.4** | **0.5** | **0.6** | **0.8** | **0.9** | **1** |
| **1 mmol/L standard (μL)** | 0 | 40 | 80 | 100 | 120 | 160 | 180 | 200 |
| **Buffer solution (μL)** | 200 | 160 | 120 | 100 | 80 | 40 | 20 | 0 |

## Sample preparation

### ① Sample preparation

**Serum and plasma:** Add serum (plasma) into 10 kDa MWCO Spin Filter and centrifuge at 12000×g for 25 min. Collect the filtrate and preserve it on ice for detection. Detect the prepared sample on the same day.

**Tissue sample:**

① Harvest the amount of tissue needed for each assay (initial recommendation 100 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 100 mg tissue in 900 μL double distilled water with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min at 4℃.

⑥ Collect the filtrate and preserve it on ice for detection. Detect the prepared sample on the same day.

**Cell samples:**

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL double distilled water with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

④ Collect supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 25 min at 4℃.

⑤ Collect the filtrate and preserve it on ice for detection. Detect the prepared sample on the same day.

## ② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|---|---|
| 10% Mouse liver tissue homogenate | 1 |
| 10% Mouse kidney tissue homogenate | 1 |
| 1×10^6 HL-60 cells | 1 |
| 1×10^6 293T cells | 1 |
| Mouse plasma | 1 |
| Human serum | 1 |
| Rat serum | 1 |

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

## Operating steps

① Standard well: Add 40 μL of different concentrations solution to standard well.

Sample well: Add 40 μL of sample to sample well.

Control well: Add 40 μL of sample to control well.

② Add 20 μL of enzyme reagent to standard well and sample well.

Add 20 μL of buffer solution to control well.

③ Add 160 μL of reaction working solution to each well

④ Mix fully with microplate reader for 5 s. Measure the OD value of each well at 340 nm with microplate reader, recorded as $A_1$. Incubated at 25°C for 10 min, measure the OD value of each well at 340 nm with microplate reader, recorded as $A_2$, $\Delta A = A_1 - A_2$ (If the OD value of the change detected by the sample was less than 0.005, the incubation time could be extended for 5-10 min for detection).

## Calculation

**The standard curve**:

1. Average the duplicate reading for each standard.

2. Subtract the mean ΔA value of the blank (Standard #①) from all standard readings. This is the absoluted ΔA value.

3. Plot the standard curve by using absoluted ΔA value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

**The sample**:

**1. Serum (plasma) sample:**

$$\text{adenosine content (mmol/L)} = (\Delta A_{340} - b) \div a \times f$$

**2. Tissue sample:**

$$\text{adenosine content (mmol/kg tissue)} = (\Delta A_{340} - b) \div a \div m \times v \times f$$

**3. Cell samples:**

$$\text{adenosine content (μmol/10^6)} = (\Delta A_{340} - b) \div a \div n \times v \times f$$

**[Note]**

$\Delta A_{340}$: $\Delta A_{340} = \Delta A_{\text{sample}} - \Delta A_{\text{control}}$, $\Delta A = A_1 - A_2$.

m: The weight of sample, kg.

n: The number of cell samples, 10^6.

v: The volume of double distilled water in the preparation step, L.

f: Dilution factor of sample before test.

# Appendix I Performance Characteristics

## 1. Parameter:

### Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol/L)** | 0.20 | 0.40 | 0.80 |
| **%CV** | 3.50 | 3.60 | 5.80 |

### Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (mmol/L)** | 0.20 | 0.40 | 0.80 |
| **%CV** | 1.9 | 5.6 | 6.8 |

### Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96.7%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| **Expected Conc. (mmol/L)** | 0.20 | 0.40 | 0.80 |
| **Observed Conc. (mmol/L)** | 0.18 | 0.38 | 0.81 |
| **recovery rate(%)** | 94.0 | 95.0 | 101.0 |

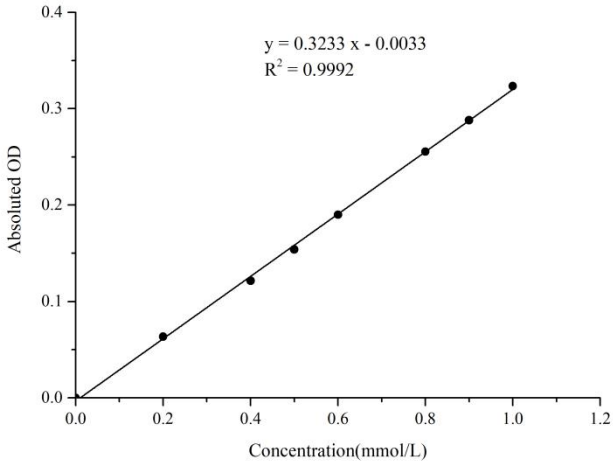
### Sensitivity

The analytical sensitivity of the assay is 0.027 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## 2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

| **Concentration (mmol/L)** | **0** | **0.2** | **0.4** | **0.5** | **0.6** | **0.8** | **0.9** | **1** |
|---|---|---|---|---|---|---|---|---|
| **$A_1$ value** | 1.873 | 1.849 | 1.816 | 1.811 | 1.788 | 1.753 | 1.745 | 1.746 |
| | 1.873 | 1.840 | 1.802 | 1.796 | 1.785 | 1.767 | 1.759 | 1.754 |
| **$A_2$ value** | 1.870 | 1.783 | 1.691 | 1.652 | 1.595 | 1.496 | 1.454 | 1.418 |
| | 1.870 | 1.773 | 1.678 | 1.641 | 1.592 | 1.507 | 1.468 | 1.429 |
| ΔA | 0.003 | 0.067 | 0.124 | 0.155 | 0.193 | 0.257 | 0.291 | 0.328 |
| | 0.003 | 0.067 | 0.125 | 0.157 | 0.193 | 0.260 | 0.291 | 0.325 |
| **Average** ΔA | 0.003 | 0.067 | 0.125 | 0.157 | 0.193 | 0.259 | 0.291 | 0.327 |
| **Absoluted** ΔA | 0 | 0.064 | 0.122 | 0.154 | 0.190 | 0.256 | 0.288 | 0.324 |



# Appendix Π Example Analysis

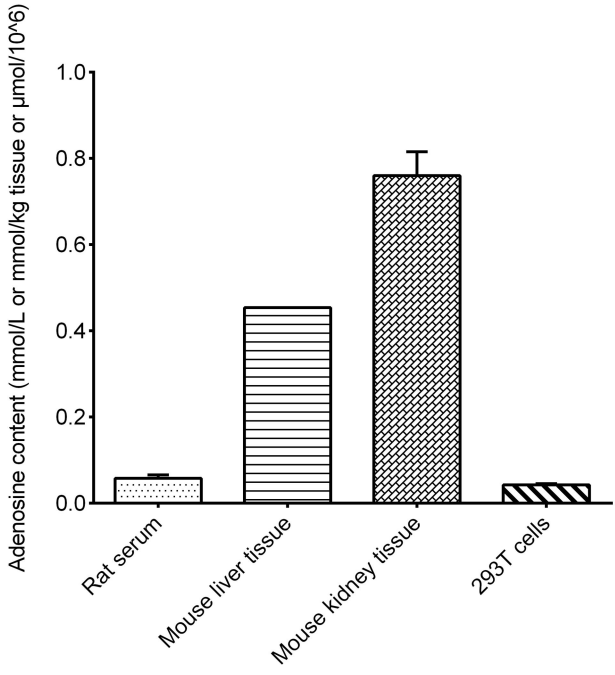
**Example analysis:**

Take 40 μL of rat serum and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 0.3233 x - 0.0033. The $A_1$ value of the sample well is 1.754, the $A_2$ value of the sample well is 1.590, $\Delta A_{sample}$ = 1.754 - 1.590 = 0.164. The $A_1$ value of the control well is 1.725, the $A_2$ value of the control well is 1.576, $\Delta A_{control}$ = 1.725 - 1.576 = 0.149, $\Delta A_{340} = \Delta A_{sample} - \Delta A_{control}$ = 0.164 - 0.149 = 0.015, and the calculation result is:

adenosine content (mmol/L) = (0.015 + 0.0033) ÷ 0.3233 = 0.057 mmol/L

Detect rat serum, 10% mouse liver tissue homogenate, 10% mouse kidney tissue homogenate, and 1×10^6 293T cells, according to the protocol, the result is as follows:



## Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.