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

Catalog No：E-BC-F009

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：2.8-50 μmol/L

Elabscience® Adenosine Diphosphate (ADP) Fluorometric 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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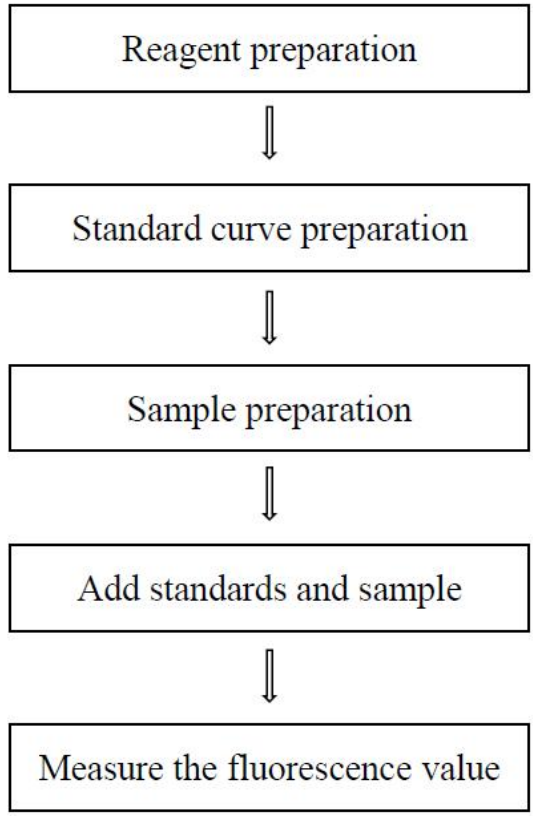
Assay summary



Intended use

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

Detection principle

Adenosine diphosphate (ADP) is a nucleoside that plays a key role in energy transfer reactions. In organisms, ADP is usually the product of Adenosine triphosphate (ATP) which hydrolysis after the loss of a phosphate group, that is breaking a high-energy phosphate bond and releasing energy. ADP exists in high-density particles in platelet cells and is released when platelets undergo coagulation reaction. ADP affects the shape and biological behavior of platelets through ADP receptors on platelets, and further accelerates the process of platelet coagulation.

The detection principle of this kit is that ADP and the substrate are catalyzed by enzyme reagents to make the chromogenic agent produce fluorescence. The fluorescence microplate reader was used to detect the excitation wavelength of 535 nm and the emission wavelength of 587nm, and the ADP content in the sample was calculated according to the standard curve.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Substrate	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months shading light
Reagent 3	Enzyme Reagent	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Chromogenic Agent	0.15 mL × 1 vial	0.3 mL × 1 vial	-20°C, 12 months shading light
Reagent 5	5 mmol/L Standard Solution	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months shading light

Reagent 6	Cell Lysis Buffer	7 mL × 1 vial	14 mL × 1 vial	-20°C, 12 months
	Black 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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator(37°C), 3 KD ultrafiltration tube

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 1 mL of buffer solution, mix well to dissolve. The substrate working solution should be prepared on spot and used up within 4 hours protected from light.

③ The preparation of measuring working solution：
Before testing, please prepare sufficient mersuring working solution according to the test wells. For example, prepare 225 μL of mersuring working solution (mix well 210 μL of buffer solution, 5 μL of substrate working solution and 10 μL of enzyme reagent). The measuring working solution should be prepared on spot and protected from light.

④ The preparation of chromogenic working solution:

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 50 μL of chromogenic working solution (mix well 46 μL of buffer solution and 4 μL of chromogenic agent). The chromogenic working solution should be prepared on spot and protected from light. The solution should be used up within 1 day.

⑤ The preparation of 50 μmol/L standard solution:

Dilute 10 μL of 5 mmol/L standard solution with 990 μL of normal saline (0.9% NaCl), mix well to dissolve. The standard solution should be prepared on spot and protected from light. The solution should be used up within 1 day.

⑥ The preparation of standard curve：

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

Dilute 50 μmol/L standard solution with normal saline (0.9% NaCl) to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 15, 20, 30, 35, 40,50 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**10**	**15**	**20**	**30**	**35**	**40**	**50**
50 μmol/L standard (μL)	0	40	60	80	120	140	160	200
Normal saline (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum, plasma sample:

① Prepare serum/plasma as the common method.

② Take 100-500 μL of sample and add it to 3 KD ultrafiltration tube. Centrifuge at 12000×g for 15 min.

③ Take the filtered sample supernatant and preserve it on ice for detection.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

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

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

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

⑤ Take 100-500 μL of supernatant and add it to 3 KD ultrafiltration tube. Centrifuge at 12000×g for 15 min.

⑥ Take the filtered sample supernatant and preserve it on ice for detection.

Cell (adherent or suspension) samples:

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL cell lysis buffer. Place on the ice box and crack for 10 min, mix well in 5 minutes.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and add it to 3 KD ultrafiltration tube. Centrifuge at 12000×g for 15 min.

⑤ Take the filtered sample supernatant and preserve it on ice for detection.

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	1-3
10% Rat kidney tissue homogenate	1-3
10% Mouse heart tissue homogenate	1
10% Rat lung tissue homogenate	1-3
Mouse plasma	1-3
Rat serum	1
1 × 10^6 Molt-4 cell	1
1.2 × 10^6 HL-60 cell	1
1 × 10^6 293T cell	1
1.3 × 10^6 CHO cell	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the well.

Sample well: add 20 μL of sample into the well.

② Add 140 μL of measuring working solution into each well.

③ Add 20 μL of chromogenic working solution into each well.

④ Mix fully with microplate reader for 5 s. Incubate at 37°C for 40 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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:

$$\text{ADP content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{ADP content (μmol/kg wet weight)} = (\Delta F - b) \div a \div (m \div V) \times f$$

3. Cell sample:

$$\text{ADP content (μmol/10^6)} = (\Delta F - b) \div a \div (n \div V) \times f$$

[Note]

ΔF: The absolute fluorescence value of sample, $F_{sample} - F_{blank}$.

f: Dilution factor of sample before tested.

m: The wet weight of sample, g.

V: The volume of homogenate medium during the preparation of tissue or cell sample, mL.

n: The number of cells, 10^6.

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 (μmol/L)	10	25	40
%CV	4.0	4.5	5.0

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 (μmol/L)	10	25	40
%CV	4.4	5.6	9.6

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	10	25	40
Observed Conc.(μmol/L)	9.5	24.0	38.8
Recovery rate (%)	95	96	97

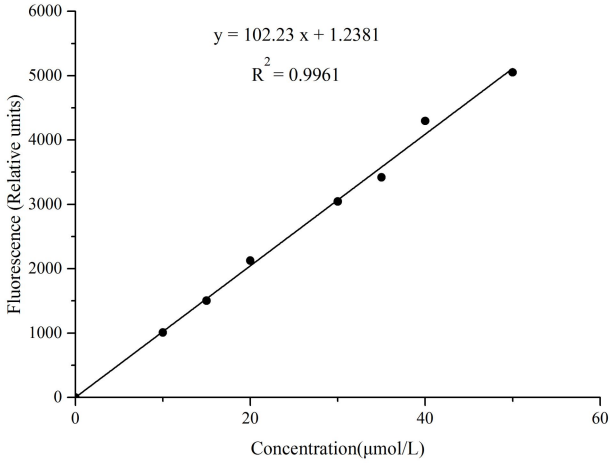
Sensitivity

The analytical sensitivity of the assay is 2.8 μmol/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 (μmol/L)	0	10	15	20	30	35	40	50
Fluorescence value	1719	2830	3324	3906	4762	4878	6056	6676
	1816	2727	3217	3886	4867	5499	6074	6963
Average fluorescence value	1768	2778	3271	3896	4814	5188	6065	6819
Absoluted fluorescence value	0	1010	1503	2128	3046	3420	4297	5051



Appendix II Example Analysis

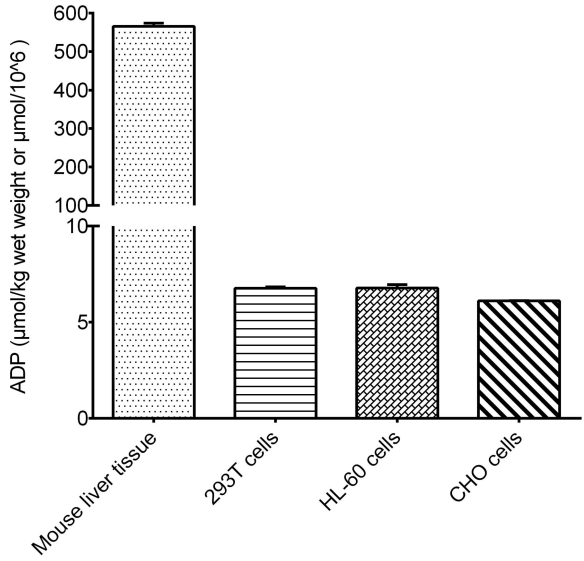
Example analysis:

Dilute 10% mouse liver tissue homogenate, dilute for 2 times, take 20 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y =102.23x + 1.2381, the average fluorescence value of the sample is 4983, the average fluorescence value of the blank is 1335, ΔF = 4983 – 1335 = 3648, and the calculation result is:

ADP content (μmol/kg wet weight) = (3648 - 1.2381) ÷ 102.23 ÷ (0.1 ÷ 0.9) × 2 = 642.10 μmol /kg wet weight

Detect 10% mouse liver tissue homogenate (dilute for 2 times), 1×10^6 293T cells, 1.2×10^6 HL-60 cells and 1.3×10^6 CHO 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.