

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

**Catalog No: E-BC-K826-M**

**Specification: 96T(40 samples)**

**Measuring instrument: Microplate reader (600-640 nm)**

**Detection range: 0.03-5.00 U/L**

## **Elabsience® ATPase/GTPase Activity 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@elabsience.com](mailto:techsupport@elabsience.com)

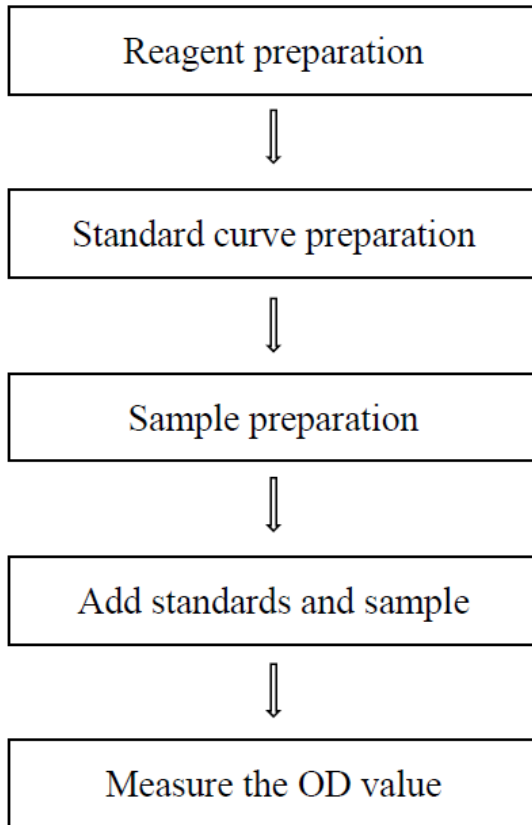
Website: [www.elabsience.com](http://www.elabsience.com)

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 detect the Adenosine Triphosphatase (ATPase) activity and Guanosine Triphosphatase (GTPase) activity in tissue, cells serum and plasma samples.

## **Detection principle**

Adenosine triphosphatase (ATPase) is a class of enzymes that catalyze the conversion of ATP into ADP and inorganic phosphate. The catalytic process releases energy and drives a variety of cellular activities, such as ion transmembrane transport and muscle contraction.

Guanosine triphosphatase (GTPase) catalyzes the conversion of GTP into GDP and inorganic phosphate. GTPases play key roles in signal transduction, protein synthesis, and cytoskeleton regulation.

This kit calculates the activity of ATPase or GTPase by measuring the change in OD value at 620 nm per unit time of the products produced by the hydrolysis of ATP or GTP catalyzed by ATPase or GTPase in the sample.

## Kit components & storage

Item	Component	Size 1(96 T)	Storage
Reagent 1	Reaction Solution	12 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	20 mmol/L ATP	1.2 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	20 mmol/L GTP	1.2 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 4	Stop Solution	17 mL × 1 vial	2-8°C, 12 months
Reagent 5	Chromogenic Agent	22 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 6	3 mmol/L Standard Solution	1.6 mL × 1 vial	2-8°C, 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid reagents with small volumes or powders, centrifuge them before use to prevent loss.

## Instruments

Microplate reader (600-640 nm, optimum wavelength: 620 nm), Incubator (37°C)

## Materials required but not provided

Double distilled water, Normal Saline (0.9% NaCl), 1×PBS (0.01 M, pH 7.4)

## Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of ATPase Working Solution:

Before testing, prepare a sufficient amount of ATPase Working Solution according to the test wells. For example, prepare 1100 µL of ATPase Working Solution (mix 1000 µL of Reaction Solution and 100 µL of 20 mmol/L ATP thoroughly). Stable for 7 days when stored at 2-8°C protected from light.

③ The preparation of GTPase Working Solution:

Before testing, prepare a sufficient amount of GTPase Working Solution according to the test wells. For example, prepare 1100 µL of GTPase Working Solution (mix 1000 µL of Reaction Solution and 100 µL of 20 mmol/L GTP thoroughly). Store at -20°C for 7 days protected from light.

④ The preparation of 150 µmol/L Standard Solution:

Before testing, please prepare sufficient 150 µmol/L standard solution according to the test wells. For example, prepare 1000 µL of 150 µmol/L standard solution (mix well 950 µL of double distilled water and 50 µL of 3 mmol/L Standard Solution). Store at -20°C for 7 days protected from light.

⑤ Standard curve preparation:

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

Dilute 150  $\mu\text{mol/L}$  standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 30, 60, 75, 90, 105, 120, 150  $\text{mmol/L}$ . Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
<b>Concentration (<math>\mu\text{mol/L}</math>)</b>	<b>0</b>	<b>30</b>	<b>60</b>	<b>75</b>	<b>90</b>	<b>105</b>	<b>120</b>	<b>150</b>
<b>150 <math>\mu\text{mol/L}</math> Standard (<math>\mu\text{L}</math>)</b>	0	40	60	80	100	140	160	200
<b>Double distilled water (<math>\mu\text{L}</math>)</b>	200	160	140	120	100	60	40	0

## Sample preparation

**Serum and plasma:** detect directly. If not detected on the same day, the serum or plasma can be stored at  $-80^{\circ}\text{C}$  for a month.

### 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  $\mu\text{L}$  normal saline (0.9% NaCl) with a dounce homogenizer at  $4^{\circ}\text{C}$ .
- ④ Centrifuge at  $10000\times g$  for 10 min at  $4^{\circ}\text{C}$  to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

## Cell sample

- ① Harvest the number of cells needed for each assay (initial recommendation  $1 \times 10^6$  cells).
- ② Wash cells with PBS (0.01 M, pH 7.4).
- ③ Homogenize  $1 \times 10^6$  cells in 200  $\mu$ L normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4°C.
- ④ Centrifuge at 10000 $\times$ g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

## Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenization	15-30
10% Mouse kidney tissue homogenization	24-50
10% <i>Epipremnum aureum</i> leaf tissue homogenization	6-15
10% <i>Schefflera octophylla</i> leaf tissue homogenization	6-15
Mouse serum	8-15
Human plasma	8-15
$1 \times 10^6$ 293T cells	2-8
$1 \times 10^6$ A549 cells	2-8

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

### Hydrolysis reaction:

- ① ATPase tube: Add 50  $\mu\text{L}$  of sample to the tubes.  
GTPase tube: Add 50  $\mu\text{L}$  of sample to the tubes.  
Control tube: Add 50  $\mu\text{L}$  of sample to the tubes.
- ② ATPase tube: Add 100  $\mu\text{L}$  of ATPase Working Solution to the tubes.  
GTPase tube: Add 100  $\mu\text{L}$  of GTPase Working Solution to the tubes.  
Control tube: Add 100  $\mu\text{L}$  of Reaction Solution to the tubes
- ③ Mix well and incubate the tubes at 37°C for 30 min protected from light.
- ④ Add 150  $\mu\text{L}$  of Stop Solution to each tube. vortex thoroughly, centrifuge at 12000 $\times$ g for 10 min at 4°C, and collect the supernatant for subsequent determination.

### Chromogenic reaction:

- ⑤ Standard well: Add 20  $\mu\text{L}$  of Standard Solution with different concentrations to the corresponding wells.  
ATPase well: After hydrolysis reaction step, add 20  $\mu\text{L}$  of solution of ATPase tube to the corresponding wells.  
GTPase well: After hydrolysis reaction step, add 20  $\mu\text{L}$  of solution of GTPase tube to the corresponding wells.  
Control well: After hydrolysis reaction step, add 20  $\mu\text{L}$  of solution of control tube to the corresponding wells.
- ⑥ Add 200  $\mu\text{L}$  of Chromogenic Agent to each well.
- ⑦ Mix thoroughly and incubate at 37°C for 30 min protected from light.
- ⑧ Measure the OD value of each well at 620 nm with microplate reader.

## Calculation

### The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the Corrected OD value.
3. Plot the standard curve by using Corrected OD 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:

#### Tissue or cell sample:

**Definition:** One unit of enzyme is defined as the amount enzyme in 1 g protein will catalyze substrate to produce 1  $\mu\text{mol}$  product per 1 min at 37 °C.

$$\text{ATPase/GTPase activity (U/gprot)} = (\Delta A_{620} - b) \div a \div T \times f$$

#### Serum and plasma sample

**Definition:** One unit of enzyme is defined as the amount enzyme in 1 L plasma or serum will catalyze substrate to produce 1  $\mu\text{mol}$  product per 1 min at 37 °C.

$$\text{ATPase/GTPase activity (U/L)} = (\Delta A_{620} - b) \div a \times \frac{V_1}{V_2} \div T \times f$$

### [Note]

$\Delta A_{620}$ : The corrected OD value of the sample ( $\text{OD}_{\text{sample}} - \text{OD}_{\text{control}}$ ).

$C_{\text{pr}}$ : The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

T: Reaction time, 30 min.

$V_1$ : Total reaction volume, 300  $\mu\text{L}$ .

$V_2$ : Sample volume, 50  $\mu\text{L}$ .

## Appendix I Performance Characteristics

### 1. Parameter:

#### Intra-assay Precision

Three 10% Mouse liver tissue homogenization samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Three 10% Mouse kidney tissue homogenization samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/gprot)	0.71	1.06	1.55
%CV	2.6	1.7	6.8

#### Inter-assay Precision

Three 10% Mouse liver tissue homogenization samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Three 10% Mouse kidney tissue homogenization samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/gprot)	0.17	0.28	0.37
%CV	6.7	9.7	10.6

## 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 98%.

	Sample 1	Sample 2	Sample 3
<b>Expected Conc. (U/gprot)</b>	4.12	6.18	9.27
<b>Observed Conc. (U/gprot)</b>	4.34	6.18	8.35
<b>Recovery rate (%)</b>	105	100	90

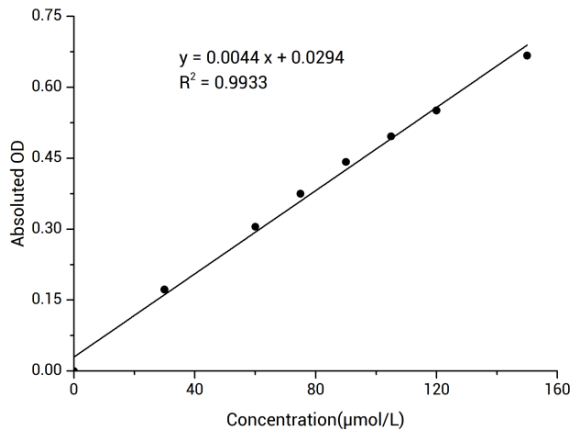
## Sensitivity

The analytical sensitivity of the assay is 0.03 U/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 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	30	60	75	90	105	120	150
OD	0.107	0.275	0.416	0.488	0.554	0.599	0.661	0.788
	0.107	0.282	0.408	0.475	0.544	0.606	0.655	0.760
Average OD	0.107	0.279	0.412	0.482	0.549	0.603	0.658	0.774
Corrected OD	0	0.172	0.305	0.375	0.442	0.496	0.551	0.667



## Appendix II Example Analysis

### Example analysis:

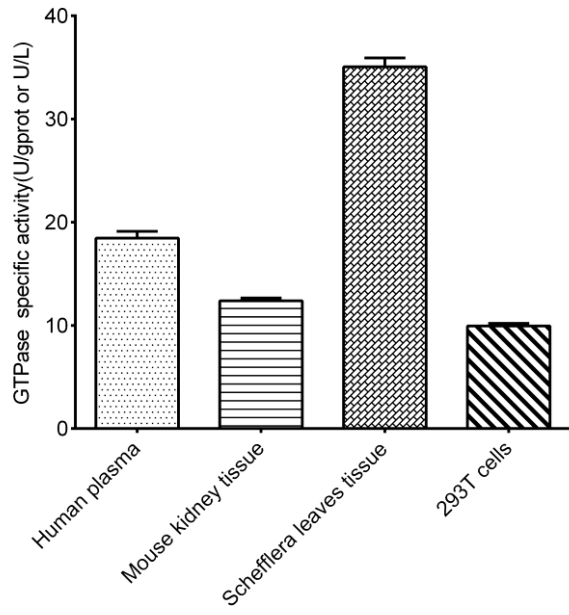
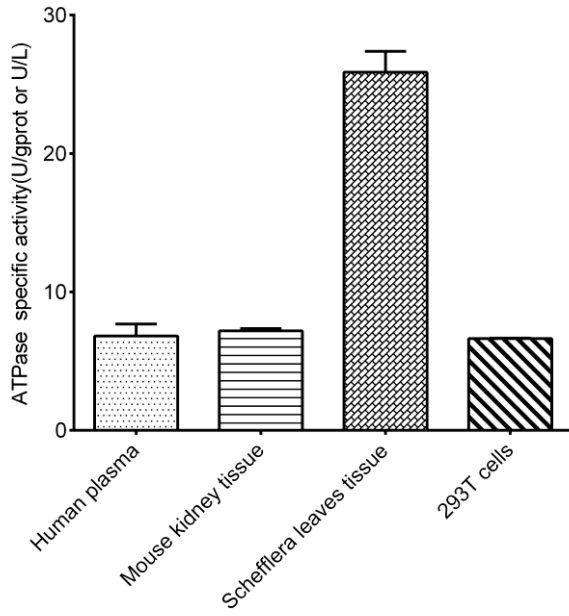
Take 50  $\mu$ L of the 40-fold diluted 10% mouse kidney tissue homogenate supernatant to the tube. Proceed according to the operating steps. The results are as follows:

standard curve:  $y = 0.0044x + 0.0294$ . The average OD value of the ATPase is 0.454, the average OD value of the GTPase is 0.665, the average OD value of the control is 0.143, the concentration of protein is 13.21 gprot/L, and the calculation result is:

$$\begin{aligned} \text{ATPase activity (U/gport)} &= (0.454 - 0.143 - 0.0294) \div 0.0044 \div 15 \times 200 \\ &\div 13.21 \times 40 \div 30 = 6.46 \text{ U/gport} \end{aligned}$$

$$\begin{aligned} \text{GTPase activity (U/gport)} &= (0.665 - 0.143 - 0.0294) \div 0.0044 \div 15 \times 200 \\ &\div 13.21 \times 40 \div 30 = 11.30 \text{ U/gport} \end{aligned}$$

Detect Human plasma (dilute for 10 times), 10% mouse kidney tissue homogenization (the concentration of protein is 13.21 gprot/L, dilute for 40 times), 10% Schefflera octophylla leaf tissue homogenization (the concentration of protein is 0.62 gprot/L, dilute for 10 times) and  $1 \times 10^6$  293T cells (the concentration of protein is 0.82 gprot/L, dilute for 4 times), 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.