

EZMeta™ $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase Colorimetric Activity Kit

Cat #: D-AKC1810

Size: 48T / 96T

Storage: Stored at -20°C for 12 months, protected from light

Product Information

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria

Assay Principle

The $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase is an enzyme widely distributed in biomembrane system of the organism. It performs several functions in cell physiology and it can catalyze ATP hydrolysis to produce ADP and inorganic Phosphorus. EZMeta™ $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase Colorimetric Activity Kit provides a simple method for detecting $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase activity in a variety of biological samples such as serum, plasma, animal and plant tissues and cells, bacteria. In the assay, $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase catalyzes ATP hydrolysis to produce ADP and inorganic phosphorus. The content of inorganic phosphorus can reflect the activity of ATPase.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4°C
Assay Buffer	5 mL	10 mL	4°C
Reagent I	1	1	-20°C, protected from light
Reagent II	1 mL	2 mL	4°C
Reagent III	1	1	4°C, protected from light
Reagent IV	1	1	4°C, protected from light
Reagent V	1	1	4°C, protected from light
Reagent VI	25 mL	25 mL	4°C
Reagent VII	5 mL	10 mL	4°C

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- Water bath
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent I : Add 6 mL deionized water before use; Store at -20°C, protected from light.

Reagent II : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent III: Add 3 mL deionized water before use; Store at 4°C, protected from light.

Reagent IV: Add 25 mL deionized water before use; Store at 4°C, protected from light.

Reagent V: Add 25 mL deionized water before use; Store at 4°C, protected from light.

Reagent VI: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent VII: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

0.5 $\mu\text{mol/mL}$ Phosphorus Standard Solution: Dilute Reagent VII by 20 times. The specific operation method is to add 0.1 mL Reagent VII to 1.9 mL deionized water, and mix well.

Phosphorus Fixing Reagent: According to the ratio of deionized water: Reagent IV: Reagent V: Reagent VI= 2:1:1:1, the prepared Phosphorus Fixing Reagent should be light yellow. If the color of the prepared reagent is colorless, the reagent is invalid, and if blue, it is phosphorus contamination. Phosphorus Fixing Reagent is freshly prepared.

Notes: The Reagent IV and Reagent V can be stored at 4°C for one week after dissolution. It is better to use new beakers, glass rods and glass pipettes, or use disposable plastic containers to prepare reagents to avoid phosphorus contamination.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Serum, Plasma, Cell Supernatant or other liquid samples: Directly test.
2. Animal and Plant Tissue samples: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize. Centrifuge at 8,000 g for 10 minutes at 4°C. Use supernatant for assay. Keep it on ice to be tested.
3. Cells or Bacteria samples: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with EZMeta™ Ca²⁺/Mg²⁺-ATPase Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.

2. Enzyme catalysis (add the following reagents to the EP tube):

Reagent	Control Tube (μL)	Test Tube (μL)
Assay Buffer	65	45
Reagent I	60	60
Reagent II	0	20
Sample	0	100
Mix well, put into water bath at 37°C (for mammals) or 25°C (for other species) for 10 min		
Reagent III	25	25
Sample	100	0
Mix well, Centrifuge at 4,000 g for 10 min at room temperature, and take the supernatant		

3. Determination of phosphorus (add the following reagents to the 96-well plate or microglass cuvette):

Reagent	Blank Well (μL)	Standard Well (μL)	Control Well (μL)	Test Well (μL)
0.5 μmol/mL Phosphorus Standard Solution	0	20	0	0
Supernatant	0	0	20	20
Deionized Water	20	0	0	0
Phosphorus Fixing Reagent	200	200	200	200

4. Mix well, keep at room temperature for 30 min and measure absorbance at 660 nm, record the optical density value of each tube, recorded as A_{Blank} , A_{Standard} , A_{Control} and A_{Test} .

Note: Every sample needs to set a control well. Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If $A_{\text{Test}} - A_{\text{Control}}$ is less than 0.001, increase the sample quantity appropriately. If $A_{\text{Test}} - A_{\text{Control}}$ is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase active in serum, plasma or other liquids samples

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase per milliliter of serum (plasma) per hour is defined as an enzyme activity unit.

$$\begin{aligned}\text{Ca}^{2+}/\text{Mg}^{2+}\text{-ATPase activity (U/mL)} &= C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \times V_{\text{Total}} \div V_{\text{Sample}} \div (T \div 60) \\ &= \mathbf{7.5 \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}})}\end{aligned}$$

2. Calculation of $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase active in tissues, bacteria or cells

(1) Calculated by protein concentration

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase per mg tissue protein per hour is defined as an enzyme activity unit.

$$\begin{aligned}\text{Ca}^{2+}/\text{Mg}^{2+}\text{-ATPase activity (U/mg)} &= C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \times V_{\text{Total}} \div (C_{\text{pr}} \times V_{\text{Sample}}) \div (T \div 60) \\ &= \mathbf{7.5 \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \div C_{\text{pr}}}\end{aligned}$$

(2) Calculated by fresh weight of samples

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase per gram of tissue per hour is defined as an enzyme activity unit.

$$\begin{aligned}\text{Ca}^{2+}/\text{Mg}^{2+}\text{-ATPase activity (U/g)} &= C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \times V_{\text{Total}} \div (V_{\text{Sample}} \div V_{\text{Sample Total}} \times W) \div (T \div 60) \\ &= \mathbf{7.5 \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \div W}\end{aligned}$$

(3) Calculated by bacteria or cells density

Active unit definition: The amount of 1 μmol inorganic phosphorus produced by $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase per 10^4 bacteria or cells per hour is defined as an enzyme activity unit.

$$\begin{aligned}\text{Ca}^{2+}/\text{Mg}^{2+}\text{-ATPase activity (U/10}^4\text{)} &= C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \times V_{\text{Total}} \div (V_{\text{Sample}} \div V_{\text{Sample Total}} \times 500) \div (T \div 60) \\ &= \mathbf{0.015 \times (A_{\text{Test}} - A_{\text{Control}}) \div (A_{\text{Standard}} - A_{\text{Blank}})}\end{aligned}$$

Where: $C_{\text{Standard well}}$: Concentration of phosphorus standard solution, 0.5 $\mu\text{mol/mL}$; V_{Total} : The total volume of enzymatic reactions, 0.25 mL; V_{Sample} : The volume of sample added to the reaction system, 0.1 mL; $V_{\text{Sample Total}}$: The volume of Extraction Buffer added to samples, 1 mL; T: Reaction time (min), 10 min; Cpr: Protein concentration of the sample,

mg/mL; W: Fresh weight of sample, g; 500: Total number of bacteria or cells, 5×10^6 .

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.