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

Mg^{2+} -EGTA-ATPase Activity Assay Kit

Catalog No.: BC00151

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Mg^{2+} -EGTA-ATPase Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Fish tissue
Assay Type	Quantitative
Detection Instrument	Spectrophotometer or Microplate reader (optimal detection wavelength 636 nm)

Product Introduction

ATPase activity can be categorized into Ca^{2+}- ATPase activity, Mg^{2+}-ATPase activity, Ca^{2+}-Mg^{2+}-ATPase activity, and Mg^{2+}- EGTA-ATPase activity. Ca^{2+}-ATPase activity is a good indicator of myosin integrity; Mg^{2+}-ATPaSe activity is an indicator of the integrity of the actomyosin complex in the presence of endogenous Ca^{2+}; Ca^{2+}-Mg^{2+}-ATPaSe activity is an indicator of the integrity of the actomyosin complex in the absence of endogenous Ca^{2+}; and Mg^{2+}-EGTA-ATPase activity indicates the integrity of the tropomyosin-troponin complex. Therefore, the ATPase activity of myofibrillar proteins is often used as an indicator of actomyosin integrity to assess the degree of denaturation of fish or surimi proteins.

Principle

Actin and actin ATPase are readily affected by Ca^{2+}, Mg^{2+}, EGTA, p-chloromercurylbenzene (PCMB), ionic strength, and pH. Therefore, selecting stringent conditions for ATPase activity determination can reflect specific protein properties. Actin and actin, which constitute fish muscle proteins, generate ADP and inorganic phosphate after cleaving the phosphate group at the end of ATP. Utilizing this, the colorimetric quantification of the generated inorganic phosphate can indicate ATPase activity.

Components

Components	Size	Storage
Reagent 1	8 mL	2~8℃
Reagent 2	3 mL	2~8℃
Reagent 3	3 mL	2~8℃
Reagent 4	Powder × 2 tubes	-20℃
Preparation of Reagent 4: Add 5 mL of double-distilled water to each vial of Reagent 4 powder and dissolve it completely.		
Reagent 5	Powder × 1 tube	2~8℃
Preparation of Reagent 5: Add 5 mL of double-distilled water to each vial of Reagent 5 and heat appropriately to dissolve.		
Reagent 6	Powder × 1 tube	2~8℃
Preparation of Reagent 6: Add 10 mL of double-distilled water to each vial of Reagent 6 and store at room temperature for 3 months. [Note]: Reagent 6 is a supersaturated solution, so it is best to use 10.3 mL of hot double-distilled water (90℃～100℃) at a water bath while stirring with a glass rod to ensure complete dissolution. If not used up in one experiment, crystals may form. Before using, you can re-dissolve the crystals by heating with a water bath while stirring with a glass rod.		
Reagent 7	3 mL	2~8℃
Preparation of Reagent 7: Add 4.5 mL of double-distilled water to each bottle of Reagent 7 and store at 4℃ for 6 months.		
Reagent 8	10 mmol/L standard phosphorus stock solution	2~8℃
Reagent 9 (Phosphorus fixative)	Solution A, 7 mL × 4 bottles	2~8℃
	Solution B, 6 mL ×4 bottles	2~8℃, away from light
[Note]: Solution B of phosphorus fixative may form a gel-like substance when stored for a long time in winter or at 4℃. It cannot be dissolved at 37℃. It can be completely dissolved by bathing it in a water bath at about 60℃ for 10 minutes. Solutions A and B should be protected from phosphorus contamination.		
Reagent 10	Terminator 60 mL × 1 bottle	RT

Consumables 1	Microplate (96 wells), 1 plate	RT
Consumables 2	Plate Sealer, 2 pieces	RT
Preparation of 0.1 μmol/mL standard phosphorus solution: When using, dilute the 10 mmol/L phosphorus stock solution 100 times, i.e., take 0.1 mL and add double-distilled water to 10 mL.		
Preparation of 0.02 μmol/mL phosphorus standard solution: When using, dilute 0.1 μmol/mL phosphorus standard solution with double-distilled water 5 times, that is, take 1 mL of 0.1 μmol/mL phosphorus standard solution and add 4 mL of double-distilled water.		
Preparation of phosphorus determination reagent: When using, add one bottle of Reagent 9 A solution to one bottle of pre-warmed Reagent 9 B solution, mix thoroughly. It needs to be prepared 0.5 hours in advance. It can be stored for at least 5 days at 2-8°C. The amount of prepared colorimetric reagent is enough for 13 tubes. (If your sample volume is small and the amount of colorimetric reagent required is small, you can prepare the colorimetric reagent yourself according to the ratio of Reagent 9 A solution: Reagent 9 B solution = 7:6. Prepare only the amount needed. (When preparing the colorimetric reagent according to the ratio, prevent phosphorus contamination. It is best to use a special pipette tip.)		

Storage

The kit has a shelf life of 6 months

Preparation before the experiment

Sample processing

Extraction of fish myofibrillar protein: Accurately weigh the fish sample, thaw naturally, and cut into small pieces. Weigh 1g of the minced meat, add 15mL of pre-cooled physiological saline as a homogenizing medium, and homogenize. Then, centrifuge at 9500rpm for 30min, collect the supernatant, add 3 times the volume of pre-cooled double-distilled water, shake well, and centrifuge again at 9500rpm for 30min. Discard the supernatant and retain the precipitate. Dissolve the precipitate in pre-cooled physiological saline, shake vigorously, and finally centrifuge at 9500rpm for 20min. Collect the supernatant, which is the myofibrillar protein solution.

Operation process

Enzymatic reaction

Reagents(μL)	Control tube	Test tube
Reagent 1	70	70
Reagent 2	20	--
Reagent 3	--	20
Reagent 4	20	20
Reagent 5	--	20
Reagent 6	20	-
Sample	--	100
Mix well and react in a 37°C water bath for 10 minutes.		
Reagent 7	50	50
Sample	100	--
Mix well, centrifuge at 3500 rpm for 10 minutes, and take 150 μL of the supernatant for phosphorus determination.		

Phosphorus reaction

Reagents(mL)	Blank tube	Standard tube	Test tube	Control tube
Double-distilled water	0.15	--	--	--
0.02 μmol /L phosphorus standard solution	--	0.15	--	--
Phosphorus fixative application solution	--	--	0.15	015
Mix well and let stand at room temperature for 2 minutes.				
Terminator	0.5	0.5	0.5	0.5
Mix well, let stand at 37°C for 5-10 minutes, and measure the absorbance of each tube at 636nm with a 0.5cm optical path and zeroed with double-distilled water. [Note]: Before colorimetry, rinse the cuvettes with tap water more than 10 times, and then rinse with double-distilled water 4-5 times to avoid phosphorus contamination.				

Result calculation

Unit definition: One unit of ATPase activity is defined as the amount of inorganic phosphorus produced by ATPase from the breakdown of ATP by 1 mg of tissue protein per hour. That is, micromoles of phosphorus/mg protein/hour (μmol Pi/mgprot/hour).

$$\underset{(\mathrm{U/mgprot})}{\mathrm{Mg^{2+}-EGTA-ATPase\ Activity}} = \frac{A_{test}-A_{control}}{A_{standard}-A_{blank}} \times C_{standard} \div \frac{T}{60} \times N \div Cpr$$

Annotation

$C_{standard}$: Standard solution concentration, 0.02 μmol/mL;

N: Dilution factor of the reaction system, 2.8;

T: Enzyme reaction time, 10 min.

Cpr : Protein concentration in tissue homogenate , mgprot /L (prot refers to protein) .

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.