ATPase Stain Kit(Calcium-Cobalt Method)

Cat: AC11765

Size: 5×50mL

Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		5×50mL	Storage
Reagent(A):Alkaline Pre-Incubation Solution		50mL	RT, avoid light
Reagent(B): Acidic Pre-Incubation Solution		50mL	RT
Reagent(C): ATPase Stain Working Solution	C1:Substrate Reserve Agent	125mg	2-8℃
	C2:Substrate Diluent	1mL	RT
	C3:Staining Buffer	50mL	RT
Reagent C1 is packaged with storage resistant dry powder. Before use, reagent C2 must be added into C1 tube and mixed to prepare Substrate Stock Solution. After sub packaging, it shall be stored at- 20 ℃. Before use, take an appropriate amount of Substrate Stock Solution and Staining Buffer to prepare ATPase Stain working solution according to the ratio of 1:49, which is ready for use.			
Reagent(D): Co Solution		50mL	RT, avoid light
Reagent(E): Chromogenic Solution		2×1mL	2-8℃ , avoid light
Reagent(F): ATPase Stain Contorl Solution		10mL	RT, avoid light

Introduction

Adenosine triphosphatase (ATPase) is a kind of hydrolase, which catalyzes the hydrolysis of ATP. According to different activators, inhibitors and enzyme location, ATPase can be divided into membrane ATPase, myosin ATPase and mitochondrial ATPase. ATPase can hydrolyze adenosine triphosphate into adenosine diphosphate and phosphoric acid. This enzyme only acts on the high energy bond between phosphoric acid and phosphoric acid, thus releasing a lot of energy. The catalytic reaction is as follows: A-P-P-P + H2O→A-P-P + H3PO4 + energy.

The principle of ATPase Stain Kit (Calcium-Cobalt Method) is that adenosine triphosphate is hydrolyzed by ATPase enzyme to adenosine diphosphate and phosphoric acid, the phosphate radical combines with calcium ion to precipitate calcium phosphate, then replaced with cobalt phosphate, and the final product is black precipitate.

Self Provide Material

1%Calcium Chloride

Protocol(for reference only)

This kit does not recommend the use of fixed sample staining. If fixation or dehydration is required, auxiliary reagents containing ethanol, methanol or acetone and phosphate components cannot be used. If the dyeing results need to be stored for a long time, they can be fixed with water-based fixative before Chromogenic reduction.

Slice Pretreatment

1. Add a little acid / alkali pre incubation solution preheated at 37 ℃ to the frozen section to cover the tissue and rewarming for 1 min. (specific acid-base incubation solution selection synchronous step 2)

2. One slice is incubated with alkaline pre-incubation solution for 15min, and the excess liquid is poured out. The other slice was incubated in acidic pre-incubation solution for 5min. After slightly decanting, it was incubated with alkaline pre-incubation solution for 30s, and the excess liquid was decanted.

Staining Steps

3. During the incubation process,Mix the Substrate Stock Solution and Staining Buffer evenly in the ratio of 1:49 to prepare ATPase Stain Working Solution, which needs to be preheated at 37 ℃ and ready to use. The dosage is 100-200ul / tissue slice according to the size of tissue.

4. Pre-incubated sections were added with preheated ATPase staining solution and incubated at 37 ℃ for 30-45min.

5. Treat with 1% calcium chloride three times, each time for 1 minute. After each treatment, pour out excess liquid and do not rinse vigorously.

6. Add an appropriate amount of Co Solution dropwise to cover the sections for 3min, and soak them with distilled water for 4 times, 3min each time.

7. Prepare the Chromogenic Working Fluid in the above process, that is, take an appropriate amount of reagent (E) and dilute it 50 times with distilled water in the fume hood, that is, the Chromogenic Working Fluid is prepared and used immediately. Incubate the sections with Chromogenic Working Solution for 1-2min until the sections are uniform dark brown. Wash with running water for 10min or soak with distilled water for 4 times, 3min each time.

8. After the slice is drained of excess water, use the preheated and melted glycerol gelatin sealing agent to seal the film, and then observe it under the optical microscope.

Result

The positive site of enzyme	Brown to Black Precipitate

Muscle FiberTypes

Muscle Fiber Types	Alkaline Pre-Incubation Solution	Acidic Pre-Incubation Solution
IType	+	+++
II_AType	+++	-
II_BType	+++	-
II_CType	+++	++

Negative Control(optional)

1. Specific Control: take adjacent sections and incubate them with reagent (F)- ATPase Stain Contorl Solution after pre-incubation.The steps after incubation are consistent with normal staining.Final compare them with the sections incubated with ATPase Staining Working Solution. The same sites of both reactions are generally nonspecific phosphate (monoester) enzyme sites, and the different sites of both reactions are the sites of ATPase activity.

2. Background Control: incubate the sections with 80 ℃ distilled water for 10 min, inactivate all enzymes, and then incubate with other tissue sections at the same time. The result should be negative.

Note

1. Do not rinse the sections with water before and after they are put into ATPase Staining Working Solution or ATPase Stain Contorl Solution.

2. To identify the type of muscle fibers, it is best to use continuous frozen sections, and pay attention to avoid drying and deformation during incubation and dyeing.

3. The prepared ATPase substrate stock solution is easy to fail. It is recommended to pack it into small parts and store it at- 20 ℃ for at least 1 year. The refrigerator at 2-8 ℃ can be stored for at least 1 month. It is recommended to use it within 3 days after it is placed at room temperature. Repeated freezing and thawing is not recommended. The prepared ATPase Staining Working Solution gradually fails with the increase of precipitation in the solution. It cannot be used when obvious white precipitation is observed at the bottom of the configuration container. It is recommended to use it within 4-6 hours after full preheating.

4. The Chromogenic Solution has corrosive and pungent smell, so it should be handled carefully in the fume hood. The Chromogenic Solution is easy to be oxidized and invalid. It can be stored for at least 6 months under

airtight conditions of 2-8 ℃. It cannot be used when the solution becomes colorless or black precipitation occurs. The color of the diluted Chromogenic Working Fluid will gradually become lighter over time. It is recommended to use it within 2 hours.

5. For your safety and health, please wear experimental clothes and disposable gloves.