三磷酸腺苷酶染色液(钙钴法)

货号：G2380
规格：5×50mL
保存：2-8℃，避光保存，有效期 6 个月。

产品组成：

名称	5×50mL	保存
试剂(A):碱性预孵育液	50mL	室温，避光
试剂(B):酸性预孵育液	50mL	室温
试剂(C): ATPase 孵育液	50mL	2-8℃，避光
试剂(D): Co 溶液	50mL	室温，避光
试剂(E): 硫化液	2×1mL	室温，避光
试剂(F): ATPase 对照液	10mL	2-8℃，避光

产品介绍：

三磷酸腺苷酶(adenosine triphosphatase，ATPase)是一种水解酶，是催化 ATP 水解的一种酶。三磷酸腺苷酶根据所用激活剂、抑制剂以及酶定位的不同分为膜性三磷酸腺苷酶、肌球蛋白三磷酸腺苷酶、线粒体三磷酸腺苷酶等。ATPase 能水解三磷酸腺苷为二磷酸腺苷和磷酸，此酶只作用于磷酸与磷酸之间的高能键，因而释放大量能量。其催化反应如下：A-P-P-P + H_2O→A-P-P + H_3PO_4 +能量。

三磷酸腺苷酶染色液(钙钴法)原理在于三磷酸腺苷酶水解三磷酸腺苷为二磷腺苷和磷酸，磷酸根与钙离子结合为磷酸钙沉淀，再被置换为磷酸钴，最终产物为黑色沉淀。

自备材料：

1%氯化钙溶液、无水乙醇、95%乙醇

操作步骤：（*仅供参考*）

1. 冰冻切片恢复室温后直接固定 3-5min 或晾干。（见注意事项 1）
2. 一张切片入碱性预孵育液孵育 15min。另一张切片入酸性预孵育液孵育 5min，碱性预孵育液孵育 30s。
3. 两张切片同时入 ATPase 孵育液，37℃孵育 30-45min。
4. 1%氯化钙冲洗 3 次，每次 1min。
5. 入 Co 溶液 3min，蒸馏水充分洗 4-5 次，流水冲洗 2min。
6. 在上述过程中配制硫化工作液，即取试剂(E)用蒸馏水稀释 50 倍，即为 ALP 硫化工作液，即配即用。切片入硫化工作液孵育 1-2min。流水洗 10min 后，入蒸馏水。
7. 切片经 95%乙醇及无水乙醇脱水。
8. 二甲苯透明，树胶封片。

染色结果：

酶所在阳性部位	黑色沉淀

肌纤维分型：

肌纤维分型	碱性预孵育液	酸性预孵育液
Ⅰ型	+	+++
$Ⅱ_A$型	+++	-
$Ⅱ_B$型	+++	-
$Ⅱ_C$型	+++	++

阴性对照(可选)：

1. 取对照切片，用试剂(F)-ATPase 对照液进行孵育，并与用 ATPase 孵育液孵育的切片进行比较。两者反应相同部位可能有非特异性磷酸(单酯)酶存在，两者不同部位才是 ATP 酶活性所在。
2. 将切片入 80℃蒸馏水 10min，再与其他组织切片同时孵育，结果应为阴性。

注意事项：

1. 固定液可使用 G2147-中性福尔马林钙固定液。不建议使用乙醇、甲醇或丙酮以及含磷酸缓冲液的固定液。速冻取材切片亦可直接染色，在 Co 溶液处理后再固定。
2. 切片入 ATPase 孵育液前后不可用水冲洗。
3. 若要鉴别肌纤维所属类型，最好用连续冰冻切片，同时注意减少切片在室温暴露的时间。
4. ATPase 孵育液、硫化液易失效，最好分成小份储存，一经开启立即使用。
5. 硫化液具有腐蚀性和刺激性气味，应小心操作。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

ATPase Stain Kit

Cat: G2380
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 Incubation Solution	50mL	2-8℃, avoid light
Reagent(D): Co Solution	50mL	RT
Reagent(E): Vulcanizing Solution	2×1mL	RT , avoid light
Reagent(F): ATPase Contorl Solution	10mL	2-8℃, 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 + H_2O→A-P-P + H_3PO_4 + energy.

The principle of ATPase Stain Kit 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.

Protocol(*for reference only*)

1. Fix the frozen section for 3-5 min or dry up by air.（See Note 1)
2. Incubate one section in Alkaline Pre-Incubation Solution at room temperature for 15 min. Incubate another section in Acidic Pre-Incubation Solution at room temperature for 5min, and then incubate in Alkaline Pre-Incubation Solution for 30s again.
3. Put the two sections in ATPase Incubation Solution at the sme time and incubate for 30-45min.
4. Wash with 1% calcium chloride three times for 1 minute each time.
5. Add the sections into Co Solution for 3min, wash fully with distilled water for 4-5 times, and rinse with running water for 2min.
6. In the above process, take the Reagent (E) and dilute it with distilled water for 50 times to prepare Vulcanizing Working Solution. It is ready to use. Then put the two sections in it and incubate for 1-2min.
7. Wash with running water for 10min and remove the sections into distilled water.
8. Dehydrate by 95% ethanol and absolute ethanol.
9. Transparent by xylene and seal with resin.

Result

The positive site of enzyme	Black Precipitate

Muscle FiberTypes

Muscle Fiber Types	Alkaline Pre-Incubation Solution	Acidic Pre-Incubation Solution
Ⅰ Type	+	+++
$Ⅱ_A$Type	+++	-
$Ⅱ_B$Type	+++	-
$Ⅱ_C$Type	+++	++

Negative Control(*optional*)

Take the control section, incubate with Reagent (F)-ATPase Contorl Solution, and compare with the section incubated in ATPase Incubation Solution. There may be nonspecific phosphatase (monoester) in the same site of the two reactions, and the activity of ATPase lies in different sites of the two reactions.

Put the section into 80 ℃ distilled water for 10min and incubate it with other tissue sections at the same time, the result should be negative.

Note

1. G2147-Neutral Formalin-Calcium Fixative, 10% can be used as fixative.Ethanol, methanol , acetone or some fixative with PB are not recommended.
2. The sections should not be washed with water before and after they are put into ATPase Incubation Solution.
3. To display the fresh tissue of muscle ATPase, must slice after frozen quickly.
4. In order to identify the type of muscle fiber, it is best to use continuous frozen sections, and pay attention to reduce the exposure time of sections at room temperature.
5. ATPase Incubation Solution and Vulcanizing Solution are easy to lose effect. It is better to store them in small parts and use them immediately once open.
6. Vulcanizing Solution has corrosive and irritating smell, pleas operate carefully.
7. For your safety and health, please wear experimental clothes and disposable gloves.