Solarbio® LIFE SCIENCES
Beijing Solarbio Science & Technology Co., Ltd
Tel: 400-968-6088
Fax: 010-56371281
Http://www.solarbio.com

ATPase 染色试剂盒(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 染色工作液	C1:底物储备剂	125mg	2-8℃
	C2:底物稀释液	1mL	室温
	C3:染色缓冲液	50mL	室温，避光
试剂 C1 为耐储干粉包装，须在使用前将试剂 C2 加入 C1 管中混匀制备底物储备液，分装后-20℃保存。 临用前取底物储备液和染色缓冲液按照 1：49 的比例制备 ATPase 染色工作液，现配现用。			
试剂(D):CO 溶液		50mL	室温，避光
试剂(E):显色液		2×1mL	2-8℃，避光
试剂(F):ATPase 对照液		10mL	室温，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。

ATPase 染色试剂盒(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，再被置换为更不易溶的磷酸重金属盐，最终产物为黑色沉淀。

自备材料：

1%氯化钙溶液

操作步骤：(*仅供参考*)

切片预处理

本试剂盒不建议使用固定后样本染色。如需固定或脱水不可使用含乙醇、甲醇或丙酮以及磷酸盐成分辅助试剂。染色结果如需长期储存可在显色还原前使用水基固定液固定。

1. 冰冻切片滴加少许 37℃预热的酸/碱预孵育液覆盖组织复温 1min。（具体酸碱孵育液选择同步骤 2）
2. 一张切片滴加碱性预孵育液孵育 15min，倾去多余液体。另一张切片入酸性预孵育液孵育 5min，稍倾去后滴加碱性预孵育液孵育 30s，倾去多余液体。

染色步骤

3. 孵育过程中按照 1：49 的比例混匀底物储备液和染色缓冲液制备 ATPase 染色工作液，置于 37℃预热，即配即用。用量根据组织大小分配 100-200ul/组织切片即可。
4. 预孵育后的切片滴加预热的 ATPase 染色工作液，37℃孵育 30-45min。
5. 1%氯化钙冲洗 3 次，每次 1min，倾去多余液体。
6. 滴加适量 Co 溶液覆盖切片 3min，蒸馏水浸洗 4 次每次 3min。
7. 在上述过程中配制显色工作液，即取适量试剂(E)在通风橱内用蒸馏水稀释 50 倍，即为显色工作液，即配即用。切片入显色工作液孵育 1-2min 至切片呈均匀黑褐色。流水洗 10min 或蒸馏水浸洗 4 次每次 3min。
8. 切片倾去多余水分后使用预热融化的甘油明胶封片剂封片后光学显微镜观察。

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染色结果：

酶所在阳性部位	棕色或黑色沉淀

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. **特异性对照:**取相邻切片，经过预孵育处理后用试剂(F)-ATPase 对照液进行孵育,孵育后步骤与正常染色一致，染色结束后与用 ATPase 染色工作液孵育的切片进行比较。两者反应相同部位一般为非特异性磷酸(单酯)酶位点，两者不同部位才是 ATP 酶活性所在。
2. **本底对照:**将切片入 80°C蒸馏水孵育 10min 灭活全部酶，再与其他组织切片同时孵育，结果应为阴性。

注意事项：

1. 切片入 ATPase 染色工作液前后不可用水冲洗。
2. 若要鉴别肌纤维所属类型，最好用连续冰冻切片，同时注意避免切片在孵育和染色过程中干燥变形。
3. 配好的 ATPase 底物储备液易失效，建议配好后分装成小份置于-20℃保存，可保存至少 1 年。2-8℃冰箱可保存至少 1 月，室温放置建议 3 天内使用完毕，不建议反复冻融。配好的 ATPase 染色工作液随着溶液中沉淀量增加而逐渐失效，配置容器底部观察到明显白色沉淀时不可使用，预热充分后建议在 4-6 小时内使用完毕。
4. 显色液具有腐蚀性和刺激性气味，应在通风橱内小心操作。显色液易被氧化失效，2-8℃密闭可保存至少 6 个月，溶液变无色或出现黑色沉淀时不可使用。稀释后的显色工作液随时间推移颜色会逐渐变浅，建议在 2 小时内使用完毕。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

本产品仅供科研使用。请勿用于医药、临床医疗、食品及化妆品用途。

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ATPase Stain Kit(Calcium-Cobalt Method)

Cat: G2380
Size: 5×50mL
Storage:2-8°C, avoid light, valid for 6 months.

Kit Components

<table>
<tr><th colspan="2">Reagent</th><th>5×50mL</th><th>Storage</th></tr>
<tr><td colspan="2">Reagent(A):Alkaline Pre-Incubation Solution</td><td>50mL</td><td>RT, avoid light</td></tr>
<tr><td colspan="2">Reagent(B): Acidic Pre-Incubation Solution</td><td>50mL</td><td>RT</td></tr>
<tr><td rowspan="3">Reagent(C): ATPase Stain Working Solution</td><td>C1:Substrate Reserve Agent</td><td>125mg</td><td>2-8°C</td></tr>
<tr><td>C2:Substrate Diluent</td><td>1mL</td><td>RT</td></tr>
<tr><td>C3:Staining Buffer</td><td>50mL</td><td>RT, avoid light</td></tr>
<tr><td colspan="4">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 °C.
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.</td></tr>
<tr><td colspan="2">Reagent(D): Co Solution</td><td>50mL</td><td>RT, avoid light</td></tr>
<tr><td colspan="2">Reagent(E): Chromogenic Solution</td><td>2×1mL</td><td>2-8°C , avoid light</td></tr>
<tr><td colspan="2">Reagent(F): ATPase Stain Contorl Solution</td><td>10mL</td><td>RT, avoid light</td></tr>
</table>

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 (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*)

Slice Pretreatment

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.

1. Add a little acid / alkali pre incubation solution preheated at 37 °C 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 °C 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 °C for 30-45min.
5. Rinse with 1% Calcium Chloride for 3 times, 1min each time, and pour out the excess liquid.
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

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(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.

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