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酸性磷酸酶染色试剂盒(偶氮偶联法)

货号：G1491
规格：4×2mL/4×10mL/4×20mL
保存：-20℃，避光保存，有效期 6 个月。

产品组成：

<table>
<tr><th colspan="2">名称</th><th>4×2mL</th><th>4×10mL</th><th>4×20mL</th><th>保存</th></tr>
<tr><td colspan="2">试剂(A): ACP 固定液</td><td>10mL</td><td>50mL</td><td>100mL</td><td>2-8℃，避光</td></tr>
<tr><td rowspan="3">试剂(B):
ACP 孵育液</td><td>B1:AS-BI 染色液</td><td>0.2mL</td><td>1mL</td><td>2mL</td><td>-20℃，避光</td></tr>
<tr><td>B2: GBC 染色液</td><td>0.02mL</td><td>0.1mL</td><td>0.2mL</td><td>-20℃，避光</td></tr>
<tr><td>B3: ACP 缓冲液</td><td>1.8mL</td><td>9mL</td><td>18mL</td><td>室温，避光</td></tr>
<tr><td colspan="6">临用前，按 B1:B2:B3=10:1:90 混合，即为 ACP 孵育液，即配即用。</td></tr>
<tr><td colspan="2">试剂(C): 苏木素染色液</td><td>2mL</td><td>10mL</td><td>20mL</td><td>2-8℃，避光</td></tr>
<tr><td colspan="2">试剂(D): 甲基绿染色液</td><td>2mL</td><td>10mL</td><td>20mL</td><td>室温，避光</td></tr>
</table>

产品介绍：

酸性磷酸酶(acid phosphatase，ACP)分布极广泛，遍布各种组织，主要存在于细胞的溶酶体内，所以常作为溶酶体标志酶。酸性磷酸酶染色液(偶氮偶联法)以萘酚 AS-BI 为底物，在酸性 pH 下被酸性磷酸酶水解释放出磷酸和萘酚，萘酚与重氮盐偶联生成有色产物，定位于细胞质中。多用于新鲜血涂片、细胞涂片、冰冻切片等，亦可用于石蜡切片。

操作步骤：(*仅供参考*)

(一)血液、细胞涂片：

1. 推片：取新鲜血液或骨髓涂片置于载玻片上，推玻片于载玻片保持30度，置于血液或细胞滴液的正前方，稍往后移与血液或细胞滴液接触使后者沿推片下缘散开，再匀速沿载玻片平面平稳向前滑动至铺满血膜为止。
2. 自然晾干，ACP固定液4℃固定30s-3min，多数情况下30-60s即可。
3. 水洗，稍微晾干(不易过分干燥)。
4. 切片入ACP 孵育液，置于37℃温箱，避光浸染45-60min，水洗。
5. 复染：苏木素染色液染色5min或甲基绿染色液染色2-3min。
6. 水洗、晾干、镜检。

(二)冰冻切片：

1. 冰冻切片回温至37℃，水中浸泡1-2min。
2. 自然晾干，ACP固定液4℃固定1-3min。
3. 水洗，稍微晾干(不易过分干燥)。
4. 切片入ACP孵育液，置于37℃温箱，避光浸染45-60min，水洗。
5. 复染：苏木素染色液染色5-8min或甲基绿染色液染色2-3min。
6. 水洗、晾干、镜检。

(三)石蜡切片：

1. 石蜡切片脱蜡5-10min，重复一次。
2. 无水乙醇5min，90%乙醇和70%乙醇各2min。水洗2min。
3. 自然晾干，ACP 固定液4℃固定30s-3min，多数情况下30-60s即可。
4. 水洗，稍微晾干(不易过分干燥)。
5. 切片入ACP 孵育液，置于37℃温箱，浸染45-60min，水洗。
6. 复染：苏木素染色液染色5-8min或甲基绿染色液染色2-3min。
7. 水洗、晾干、镜检。

染色结果：

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阳性颗粒	紫红色
细胞核	蓝色(苏木素)或绿色(甲基绿)

临床意义：

1. 毛细胞白血病的毛细胞 ACP 染色呈强阳性或中度阳性，且不被酒石酸抑制。
2. 急性白血病幼单核细胞 ACP 染色呈阳性，原淋巴细胞呈弱阳性，原粒细胞对 ACP 反应不一。
3. T 淋巴细胞 ACP 染色呈阳性，颗粒粗大、分布密集。B 淋巴细胞呈阴性或颗粒细小的弱阳性。
4. 戈谢细胞呈强阳性，尼曼-皮克细胞呈阴性或弱阳性。

注意事项：

1. ACP 孵育液易失效，本法宜用皮肤穿刺血涂片，晾干后应及时染色
2. 对冰冻切片染色时，应减少切片在室温暴露的时间。
3. 样本需新鲜，取材后应立即处理，否则会影响酶的活性。
4. 组织固定需在 4℃冰箱进行，时间不宜超过 24h，否则酶活性会减弱或消失。
5. 组织在石蜡包埋时，温度不宜高于 56℃。应使用熔点为 52-54℃的石蜡进行浸蜡，浸蜡时间要短，否则酶活性会减弱或消失。

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Acid Phophatase Stain Kit (Kaplow's/Azo Coupling Method)

Cat: G1491
Size: 4×2mL/4×10mL/4×20mL
Storage: -20℃, avoid light, valid for 6 months.

Kit Components

Reagent		4×2mL	4×10mL	4×20mL	Storage
Reagent(A): ACP Fixative		10mL	50mL	100mL	2-8℃, avoid light
Reagent(B): ACP Incubation Solution	B1:AS-BI Buffer	0.2mL	1mL	2mL	-20℃, avoid light
	B2: GBC Solution	0.02mL	0.1mL	0.2mL	-20℃, avoid light
	B3: ACP Buffer	1.8mL	9mL	18mL	RT, avoid light
Mix reagent B1, B2, B3 in 10:1:90 ratio as ACP Incubation Solution before use.					
Reagent(C): Hematoxylin Solution		2mL	10mL	20mL	2-8℃, avoid light
Reagent(D): Methyl Green Solution		2mL	10mL	20mL	RT, avoid light

Introduction

Acid phosphatase (ACP) is widely distributed in various tissues, mainly in the lysosome, so it is often used as a lysosome marker enzyme. The acid phosphatase outside of lysozyme exists in endoplasmic reticulum and cytoplasm. Acidic phosphatase is different in all kinds of animals. The optimum pH for its activity is 4.5-5.5.

The reaction principle of Acid Phophatase Stain Kit (Kaplow's/Azo Coupling Method) is that phosphoric acid and naphthol are released by hydrolysis of acid phosphatase with AS-BI as substrate at acidic pH. Naphthol was coupled with diazo salts to form colored products, which were localized in the cytoplasm. It usually used for fresh blood smear, cell smear, frozen section, etc.

Protocol (*for reference only*)

For Blood or Cell Smear

1. Dry blood or cell smear in air then fix in ACP Fixative at 4 ℃ for 30s-3mins and usually for 30-60s.
2. Wash with distilled water and slightly dry in air.
3. Add the ACP Incubation Solution to sections and place in 37℃ incubator and incubate in dark for 45-60min. Then wash with distilled water.
4. Re-dyeing with Hematoxylin Solution or Methyl Green Solution for 2-3min.
5. View the sections under microscope after washing or sealing.

For Frozen Section

1. Restore the section to 37℃ by immerse in water for 1-2min.
2. Dry in air and fix in ACP Fixative for 1-3min at 4℃.
3. Wash with distilled water and slightly dry in air.
4. Add the ACP Incubation Solution to sections and place in 37℃ incubator and incubate in dark for 45-60min. Then wash with distilled water.
5. Re-dyeing with Hematoxylin Solution or Methyl Green Solution for 2-3min.
6. View the sections under microscope after washing or sealing.

For Paraffin Section

1. Dewax paraffin sections and rehydrate in graded alcohol.
2. Wash with distilled water and slightly dry in air.
3. Add the ACP Incubation Solution to sections and place in 37℃ incubator and incubate in dark for 45-60min. Then wash with distilled water.
4. Re-dyeing with Hematoxylin Solution or Methyl Green Solution for 2-3min.
5. View the sections under microscope after washing or sealing.

Result

Positive Site	Purplish Red
Nucleus	Blue or Green

Clinical Significance

1. ACP staining of hair cells in hairy cell leukemia is strongly or moderately positive, and is not inhibited by

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tartaric acid.

2. ACP staining is positive in immature monocytes of acute leukemia, and weak positive in prolymphocytes. The response of progranulocytes to ACP is different.
3. Tlymphocyte ACP staining is positive, with large and densely distributed granules. B lymphocyte is negative or weak positive with small granules.
4. Gaucher cells are strongly positive and Niemann-Pick cells are negative or weak positive.

Note

1. ACP Incubation Solution is easy to lose effect. It's recommended to use skin puncture blood smears and stain quickly after drying.
2. When staining frozen sections, the exposure time of sections at room temperature should be reduced.
3. Samples should be fresh and treated immediately after sampling, otherwise the enzyme activity will be affected.
4. Tissue should be fixed in refrigerator at 4℃ no more than 24 h, otherwise the activity of enzyme will be weakened or disappeared.
5. When embedding the tissue in paraffin, the temperature should not be higher than 56℃. It's recommended to use paraffin wax with melting point of 52-54℃ for wax soaking. The soaking time should be short, otherwise the enzyme activity will weaken or disappear.
6. For your safety and health, please wear experimental clothes and disposable gloves.

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