V02

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：

名称		4×2mL	4×10mL	4×20mL	保存
试剂(A): ACP 固定液		10mL	50mL	100mL	2-8℃，避光
试剂(B): ACP 孵育液	B1:AS-BI 染色液	0.2mL	1mL	2×1mL	-20℃，避光
	B2: GBC 染色液	0.02mL	0.1mL	0.2mL	-20℃，避光
	B3: ACP 缓冲液	1.8mL	9mL	18mL	室温，避光
临用前，按 B1:B2:B3=10:1:90 混合，即为 ACP 孵育液，即配即用。					
试剂(C): 苏木素染色液		2mL	10mL	20mL	2-8℃，避光
试剂(D): 甲基绿染色液		2mL	10mL	20mL	室温，避光
试剂 C、D 均为复染液，染色过程中择一使用即可，不建议重复染色。					

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) 样本处理：

1、 细胞涂片：取新鲜全血或骨髓制备涂片，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均匀铺开为止，自然晾干。
2、 贴壁培养细胞、细胞爬片：吸去培养基，1×PBS 清洗 3-4 次，沥去多余水分。取出细胞爬片。
3、 冰冻切片：从冰箱取出放置恢复室温，浸入蒸馏水清洗 1-2min，沥去多余水分。
4、 石蜡切片：石蜡切片脱蜡 5-10min，重复一次。无水乙醇 5min，90%乙醇和 70%乙醇各 2min，蒸馏水浸洗 2min，沥去多余水分。

(二) 染色孵育：

1、 使用 2-8℃预冷的 ACP 固定液固定 30s-3min，多数情况下 30-60s 即可。
2、 蒸馏水洗，沥去多余水分(不宜过分干燥)。
3、 （切片处理）滴加 ACP 孵育液覆盖切片，置于湿盒内 37℃温箱孵育 45-60min，蒸馏水洗。
4、 （贴壁细胞）滴加 ACP 孵育液覆盖细胞，置于 37℃温箱孵育 45-60min，蒸馏水洗。
5、 复染：苏木素或甲基绿复染液染色 2-3min。如选用苏木素染色液复染，染色后须自来水返蓝 10min 或 1×PBS 返蓝 3-5min。

(三) 封片观察：

1、 （切片处理）蒸馏水稍洗，沥去多余水分，滴加水性封片剂封片后镜检观察。
2、 （贴壁细胞）蒸馏水稍洗，可带水或 1×PBS 镜下观察。

染色结果：

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。

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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. 该染色试剂盒为酶活原位染色试剂盒，反应底物和染料易失效，收到产品后建议第一时间根据说明书描述温度妥善保存。
2. 对冰冻切片染色时，应减少切片在室温暴露的时间。
3. 酶活易受多种因素影响导致衰减或消失。建议样本新鲜取材，使用预冷固定液在 2-8℃冰箱固定，时间不宜超过 24h。包埋时推荐使用低熔点蜡（52-56℃），避免热失活。
4. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

V02

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	2×1mL	-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
Reagents C and D are both re staining solutions, and one can be used during the staining process. Repeated staining is not recommended.					

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*)

Sample Processing

1. Cell smear: Take fresh whole blood or bone marrow to prepare a smear, place the slide at 30 degrees on the slide, directly in front of the blood or cell droplet, move it back slightly to contact the blood or cell droplet, and make the latter scatter along the lower edge of the slide. Then, slide it smoothly forward along the slide plane until evenly spread, and let it dry naturally.
2. Adherent culture of cells and cell creep: remove the culture medium, wash with 1×PBS 3-4 times, and drain excess water. Remove the cell slides.
3. Frozen slice: Remove from the refrigerator and place at room temperature. Soak in distilled water and wash for 1-2 min, then drain excess water.
4. Paraffin section: Paraffin sectioning is dewaxed for 5-10 min and repeated once. Anhydrous ethanol for 5 min, 90% ethanol and 70% ethanol for 2 min each, soak in distilled water for 2 min, and drain excess water.

Staining Incubation

1. Fix with pre-cooled ACP Fixative at 2-8 ℃ for 30s-3min, and in most cases, 30-60s is sufficient.
2. Wash with distilled water and drain excess water (not too dry).
3. (Slicing treatment) Drip ACP incubation solution onto the slices and incubate them in a wet box at 37 ℃ for 45-60 min. Wash with distilled water.
4. (Adherent cells) Drip ACP incubation solution to cover the cells, incubate at 37 ℃ for 45-60 min, and wash with distilled water.
5. Re staining: Stain with Hematoxylin Solution or Methyl Green Solution for 2-3 min. If Hematoxylin Solution is used for re staining, must return blue with tap water for 10 min or with 1×PBS for 3-5 min after staining.

Sealing observation

1. (Slicing treatment) Wash slightly with distilled water, drain excess water, add water-based sealing agent dropwise, seal the film, and observe under a microscope.
2. (Adherent cells) Wash slightly with distilled water and observe under a microscope with water or 1×PBS.

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Result

Positive Site	Purplish Red
Nucleus	Blue(Hematoxylin) or Green(Methyl Green)

Clinical Significance

1. ACP staining of hair cells in hairy cell leukemia is strongly or moderately positive, and is not inhibited by 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. This staining kit is an enzyme active in situ staining kit. The reaction substrate and dye are prone to failure, and it is recommended to store the product properly according to the temperature described in the instruction manual as soon as received.
2. When staining frozen sections, the exposure time of sections at room temperature should be reduced.
3. Enzyme activity is easily affected by various factors, leading to attenuation or disappearance. It is recommended to take fresh samples and use pre cooled fixing solution to fix them in a refrigerator at 2-8 ℃ for no more than 24 hours. It is recommended to use low melting point wax (52-56 ℃) during embedding to avoid thermal deactivation.
4. For your safety and health, please wear laboratory clothes and disposable gloves for operation.