V02

抗酒石酸酸性磷酸酶染色试剂盒

货号：G1492
规格：4×10mL/4×20mL
保存：-20℃，避光保存，有效期 3 个月。

产品组成：

名称		4×10mL	4×20mL	保存
试剂(A): TRAP 固定液		50mL	100mL	2-8℃，避光
试剂(B): TRAP 孵育液	B1: AS-BI 染色液	1mL	2×1mL	-20℃，避光
	B2: GBC 染色液	0.1mL	0.2mL	-20℃，避光
	B3: TRAP 缓冲液	9mL	18mL	室温，避光
临用前，按 B1:B2:B3=10:1:90 混合，即为 TRAP 孵育液，即配即用。				
试剂(C): 苏木素复染液		10mL	20mL	2-8℃，避光
试剂(D): 甲基绿复染液		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。溶酶体外的酸性磷酸酶存在于内质网和胞质内。各种动物中的酸性磷酸酶各有不同，酸性磷酸酶的适宜 pH 为 4.5-5.5。存在于正常人肺泡巨噬细胞和白血病人脾脏的抗酒石酸酸性磷酸酶(Tartrate-resistnt acid phosphatase，TRAP)均在细胞滤泡中，并不是释放入血液。血液中的 TRAP 绝大多数来源于破骨细胞，因此可以通过测量血液中的 TRAP 了解破骨细胞的功能状态。

抗酒石酸酸性磷酸酶染色试剂盒显色原理与酸性磷酸酶染色基本相同，在常规染色基础上引入了抗酒石酸盐进行酶亚型筛选，细胞或组织切片上仅抗酒石酸酸性磷酸酶活性位点着色，常规酸性磷酸酶位点不着色。该染色液可用于新鲜血涂片、细胞涂片，亦可用于冰冻切片、石蜡切片。

自备材料：

蒸馏水、恒温箱、载玻片、光学显微镜

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 TRAP 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 TRAP 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 TRAP 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。

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染色结果：

阳性细胞胞质	紫红色
细胞核	蓝色(苏木素)或绿色(甲基绿)

常见阳性对照：

1、 肺驻留巨噬细胞通常呈 TRAP 阳性，其冰冻或石蜡切片可作为阳性对照切片。
2、 多毛细胞白血病个体外周血中多毛细胞呈 TRAP 阳性。
3、 经酶保护固定和脱钙的骨样本中，破骨细胞和破软骨细胞呈 TRAP 阳性。

注意事项：

1、 该染色试剂盒为酶活原位染色试剂盒，常用于破骨细胞细胞诱导培养后细胞鉴定和组织中特定细胞定位，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、 试剂 B2 为偶氮染料储备液，有颗粒状悬浮物属正常现象不影响染色。如担心，预混合 B1 和 B2 可使颗粒物完全溶解，但需在 2 小时内使用完毕。
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

Tartrate-Resistant Acid Phosphatase (TRAP) Stain Kit

Cat: G1492
Size: 4×10mL/4×20mL
Storage: -20℃,avoid light,valid for 3 months.

Kit Components

Reagent		4×10mL	4×20mL	Storage
Reagent(A): TRAP Fixative		50mL	100mL	2-8℃, avoid light
Reagent(B): TRAP Incubation Solution	B1: AS-BI Buffer	1mL	2×1mL	-20℃, avoid light
	B2: GBC Solution	0.1mL	0.2mL	-20℃, avoid light
	B3: TRAP Buffer	9mL	18mL	RT, avoid light
Mix reagent B1,B2,B3 in 10:1:90 ratio as TRAP Incubation Solution before use and not store for long.				
Reagent(C): Hematoxylin Solution		10mL	20mL	2-8℃, avoid light
Reagent(D): Methyl Green Solution		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. Tartrate-resistant acid phosphatase (TRAP), which mainly exists in normal human alveolar macrophages and leukemia human spleen cells, is released into the cell follicles rather than into the blood. The majority of TRAP in blood comes from osteoclasts, so we can know the function of osteoclasts by measuring TRAP in blood.

The reaction principle of Tartrate-Resistant Acid Phosphatase (TRAP) Stain Kit is basically the same as that of acid phosphatase staining. On the basis of conventional staining, tartrate resistant acid phosphatase was introduced for enzyme subtype screening. Only tartrate resistant acid phosphatase active sites were stained on cell or tissue sections, while conventional acid phosphatase sites were not stained. This staining solution can be used for fresh blood and cell smears, as well as for frozen sections and paraffin sections.

Self Provided Materials

Distilled Water, Incubator, Slide, Microscope

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 TRAP 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 TRAP 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 TRAP 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

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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.

Result

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

Clinical Significance

1. Lung resident macrophages are usually TRAP positive, and their frozen or paraffin sections can be used as positive control sections.
2. Multiple hairy cells in peripheral blood of individuals with polycystic leukemia showed TRAP positivity.
3. In the bone samples fixed and decalcified by enzyme protection, osteoclasts and chondroclasts showed TRAP positivity.

Note

1. This staining kit is an enzyme active in situ staining kit commonly used for cell identification and specific cell localization in tissues after osteoclast induction culture. 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. Reagent B2 is a reserve solution for azo dyes, and the presence of granular suspensions is a normal phenomenon that does not affect dyeing. If you are concerned, pre mixing B1 and B2 can completely dissolve the particulate matter, but it needs to be used within 2 hours.
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.