V02

碱性磷酸酶染色试剂盒(偶氮偶联法)

货号：G1480
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): ALP 固定液		2mL	10mL	20mL	室温，避光
试剂(B): ALP 孵育液	B1: AS-BI 染色液	1mL	5mL	10mL	-20℃，避光
	B2: FBB 染色液	1mL	5mL	10mL	-20℃，避光
临用时，取 B1:B2 =1:1 比例混合，即为 ALP 孵育液，即配即用。					
试剂(C):核固红复染液		2mL	10mL	20mL	2-8℃，避光
试剂(D):甲基绿复染液		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：

碱性磷酸酶(Alkaline phosphatase，简称 ALP 或 AKP)为一类磷酸酯酶，广泛分布于哺乳动物组织内，其活性所需最适 pH 9.2~9.8。此酶主要存在于物质交换活跃之处(细胞膜)，如肠上皮和肾近曲小管的刷状缘、附睾上皮之静纤毛、肝的毛细胆管膜以及微动脉和毛细血管动脉部之内皮，还见于内质网、高尔基复合体、吞饮小泡、肠上皮之溶酶体、中性粒细胞之中性颗粒以及平滑肌之细胞膜。

碱性磷酸酶染色试剂盒(偶氮偶联法)不是采用金属沉淀法来显示碱性磷酸酶活性，而是采用偶氮偶联法(又称同时偶联法)，其原理是在 pH9.2-9.8 的碱性条件下，细胞内碱性磷酸酶可使 AS-BI 磷酸盐水解，释放出磷酸与萘酚，后者与偶联重氮盐生成有色产物，定位于细胞质中。该染液可用于血液、骨髓或细胞涂片、冰冻切片等的碱性磷酸酶染色，不固定速冻切片可以最大程度上保留酶活，石蜡切片酶活保存不佳，不建议制备石蜡切片染色。碱性磷酸酶活性部位呈蓝色，位于胞浆，结果较金属盐沉淀法可靠。

自备材料：

载玻片、湿盒、普通光学显微镜

操作步骤：(*仅供参考*)

试剂(C): 核固红染色液可能会由于絮凝产生悬浮物或少量沉淀，建议取上清使用或沸水浴 5-10min 后晾至 30-40℃使用。（见注意事项 3）

(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。

(二) 染色孵育：

1、 使用 2-8℃预冷的 ALP 固定液固定 30s-3min，多数情况下 30-60s 即可。（*见注意事项 2*）
2、 蒸馏水洗，沥去多余水分(不宜过分干燥)。
3、 （切片处理）滴加 ALP 孵育液覆盖切片，置于湿盒内 37℃温箱孵育 15-20min，蒸馏水洗。
4、 （贴壁细胞）滴加 ALP 孵育液覆盖细胞，置于 37℃温箱孵育 15-20min，蒸馏水洗。
5、 复染：核固红或甲基绿复染液染色 3-5min，对于冰冻切片，一般染色 1-2min 即可。

(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：

ALP 活性部位	蓝色
细胞核	红色(核固红)或绿色(甲基绿)

血液、骨髓涂片结果判断：

一般以积分报告结果，根据 100 个中性粒细胞阳性颗粒进行 0-4 计分。

细胞分值	染色特点
0	无颗粒
1	稍有颗粒
2	中等程度颗粒
3	多数颗粒
4	充满颗粒

注意事项：

1. 血液或骨髓细胞涂片或其他样本均应新鲜，薄厚适宜，及时固定，否则会影响酶的活性。
2. 对于培养细胞和细胞爬片，如果固定后有细胞漂浮现象，可以使用 4%多聚甲醛代替 ALP 固定液进行固定处理，室温固定 10-15min。
3. 试剂(C): 核固红染色液为胶体性质溶液，低温（低于 25℃）保存或长期储存由于絮凝产生悬浮物或少量沉淀，属于正常现象，一般不影响使用。如移液器吸取观察到明显浑浊，可拧紧瓶盖沸水浴 5-10min 重新制备分散均匀的胶体溶液来恢复使用。
4. 培养细胞染色操作过程中，清洗、染色等步骤都应轻微，以免损伤或丢失细胞。
5. ALP 孵育液易失效或降低阳性强度，即配即用，不宜久置。
6. 复染时，核固红染色液或甲基绿染色液二者取其一。
7. 每次染色时，应有阳性对照片。

相关文献:

[1] Yu Jiang,Dantian Zhu,Wenfeng Liu,et al. Hedgehog pathway inhibition causes primary follicle atresia and decreases female germLine stem cell proliferation capacity or stemness. Stem Cell Research & Therapy. July 2019. (IF 4.627)

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V02

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

Cat: G1480
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) : ALP Fixative		2mL	10mL	20mL	RT, avoid light
Reagent(B) :ALP Incubation Solution	B1:AS-BI Solution	1mL	5mL	10mL	-20℃, avoid light
	B2:FBB Solution	1mL	5mL	10mL	-20℃, avoid light
Mix B1:B2 in 1:1 ratio as ALP Incubation Solution.It's ready to use.					
Reagent(C) :Nuclear Fast Red Solution		2mL	10mL	20mL	2-8℃, avoid light
Reagent(D) :Methyl Green Solution		2mL	10mL	20mL	RT, avoid light

Introduction

Alkaline phosphatase (ALP or AKP) is widely distributed in mammalian tissues. The optimum pH for its activity is 9.2-9.8. This enzyme mainly exists in active substance exchange sites (cell membranes):Such as the brush-like margin of intestinal epithelium and proximal convoluted tubule of kidney, the stationary cilia of epididymis epithelium, the capillary cholangium of liver, and the endothelium of arterioles and capillary arteries. It is also found in the endoplasmic reticulum, Golgi complex, swallowing vesicles, lysosomes of intestinal epithelium, neutral granules of neutrophils and smooth muscle cell membranes.

Alkaline Phophatase Staining Kit (Kaplow Method) is not a metal precipitation method to display alkaline phosphatase activity, but an azo coupling method (also known as simultaneous coupling method).The principle is that under the alkaline condition of pH 9.2-9.8, intracellular alkaline phosphatase can hydrolyze AS-BI phosphate and release phosphoric acid and naphthol, which can form colored products with coupling diazo salts and locate in the cytoplasm.The dye can be used for blood, bone marrow or cell smears, frozen and paraffin sections.Unsettled frozen sections can preserve enzyme activity to the greatest extent possible, while paraffin sections have poor preservation of enzyme activity, and it is not recommended to prepare paraffin sections for staining.The alkaline phosphatase positive site is blue and located in the cytoplasm. This method is more reliable than metal salt precipitation method.

Self Provided Materials

Glass slide, Wet box, Optical microscope

Protocol(*for reference only*)

Reagent C :Nuclear Fast Red Solution may produce suspended solids or a small amount of precipitation due to flocculation. It is recommended to take supernatant or boil water bath for 5-10min and then air it to 30-40 ℃. (see Note 3)

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.

Staining Incubation

1. Fix with pre-cooled ALP 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 ALP incubation solution onto the slices and incubate them in a wet box at 37 ℃ for 15-20 min. Wash with distilled water.
4. (Adherent cells) Drip ALP incubation solution to cover the cells, incubate at 37 ℃ for 15-20 min, and wash with distilled water.
5. Re staining: Stain with Nuclear Fast Red Solution or Methyl Green Solution for 3-5 min. For frozen section,

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1-2min is sufficient.

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.
1.

Result

Positive site of ALP	Blue
Nucleus	Red or Green

Criteria for blood and bone marrow smears judging

Judge according to 100 neutrophil-positive granules and report the level in 0-4

Positive level	Dyeing Characteristics
0	No Granule
1	Slightly Granule
2	Medium-Grade Granule
3	Majority Granule
4	Full Granule

Note

1. Blood or bone marrow smears or other samples should be fresh.Cut sections in appropriate thickness and fix it in time to avoid reducing activity of the enzyme.
2. For cultured cells and cell slides, if there is cell floating phenomenon after fixation, 4% paraformaldehyde can be used instead of ALP fixation solution for fixation treatment, and fixed at room temperature for 10-15 minutes.
3. Reagent C :Nuclear Fast Red Solution is a colloidal solution, which is stored at low temperature (lower than 25 ℃) or stored for a long time. Suspended solids or a small amount of precipitation are generated due to flocculation, which is a normal phenomenon and generally does not affect the use. If the colloid solution is evenly dispersed in the boiling bath, tighten the bottle cap for 5-10min to recover the turbid solution.
4. In the process of cultured cells staining, slightly wash and stain to avoid damage or loss of cells.
5. ALP Incubation Solution is easy to lose effect. Use it as soon as possible.
6. Re-dyeing by Nuclear Fast Red Solution or Methyl Green Solution.
7. There should be a positive control for each sample.

Reference

[1] Yu Jiang,Dantian Zhu,Wenfeng Liu,et al. Hedgehog pathway inhibition causes primary follicle atresia and decreases female germLine stem cell proliferation capacity or stemness. Stem Cell Research & Therapy. July 2019. (IF 4.627)

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