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

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、梯度入水后的石蜡切片等的碱性磷酸酶染色，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 2）

一、 涂片或切片

1. 血液、骨髓或细胞涂片、冰冻切片、脱蜡后石蜡切片入ALP固定液处理3min。
2. 蒸馏水稍洗 5-10s。
3. 滴加配制好的 ALP 孵育液，放入湿盒中，避光孵育 15-20min，水洗。
4. 入核固红染色液或甲基绿染色液复染 3-5min。
5. 水洗、镜检或甘油明胶封固后镜检。

二、 贴壁培养细胞

1. 取 6 孔板或其他容器培养的细胞，弃液，PBS 清洗干净。
2. 加入 ALP 固定液固定 3min 或 4%多聚甲醛固定 10-15min，蒸馏水清洗。
3. 滴加配制好的 ALP 孵育液，放入湿盒中，避光孵育 15-20min，蒸馏水清洗。
4. 入核固红染色液或甲基绿染色液复染 3～5min。
5. PBS 清洗、镜检。

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. 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。
3. 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。
4. ALP 孵育液易失效或降低阳性强度，即配即用，不宜久置。
5. 复染时，核固红染色液或甲基绿染色液二者取其一。
6. 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)

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.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 2)

For Smear or Section Stain

1. Fix blood, bone marrow or cell smears, frozen sections and dewaxed paraffin sections in ALP Fixative for 3min and wash with distilled water.
2. Add the ALP Incubation Solution to sections and place in a wet box. Incubate in dark for 15-20min and wash by distilled water.
3. Re-dyeing with Nuclear Fast Red Solution or Methyl Green Solution for 3-5min.
4. View the sections under microscope after washing or glycerol gelatin sealing.

For Adherent Cells Stain

1. Take the sample cultured in 6-well plates or other containers, remove the cell culture medium and rinse the cells completely with PBS.
2. Fix by ALP Fixative for 3mins or 4% PFA for 10-15min, then wash with PBS.
3. Add the ALP Incubation Solution to sections and place in a wet box.Incubate in dark for 15- 20min and wash with PBS.
4. Re-dyeing with Nuclear Fast Red Solution or Methyl Green Solution for 3-5min.
5. Wash with PBS and view the sections under microscope.

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. 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.
3. In the process of cultured cells staining, slightly wash and stain to avoid damage or loss of cells.
4. ALP Incubation Solution is easy to lose effect. Use it as soon as possible.
5. Re-dyeing by Nuclear Fast Red Solution or Methyl Green Solution.
6. 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)