

碱性磷酸酶-PAS 染色试剂盒

货号: G2450

规格: 6×50mL

保存: 2-8°C, 避光保存, 有效期 6 个月。

产品组成:

名称	6×50mL	保存
试剂(A): ALP 孵育液	50mL	2-8°C, 避光
试剂(B): Co 溶液	50mL	室温, 避光
试剂(C): ALP 硫化溶液	2×1mL	室温, 避光
试剂(D): 氧化剂	50mL	2-8°C, 避光
试剂(E): Schiff 试剂	50mL	2-8°C, 避光
试剂(F): 亚硫酸溶液	50mL	室温, 避光
试剂(G): ALP 对照液	10mL	2-8°C, 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膜之内皮。此酶还见于内质网、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及吞饮小泡。1946 年 McManus 最先使用 PAS 法显示黏蛋白, 该法常用来显示糖原和其他多糖。氧化剂能氧化糖类及有关物质中的 1,2-乙二醇基使之变为二醛, 醛与 Schiff 试剂能结合成一种品红化合物, 产生紫红色。由于氧化剂还可氧化细胞内其他物质, 使用时应注意选择好浓度和氧化时间, 使氧化控制在既能把乙二醇基氧化成醛基又不至于过氧化。

ALP-PAS 联合染色试剂盒不仅能够显示碱性磷酸酶活性位点和糖原等物质, 亦能区分二者。冰冻切片及石蜡切片均可。

自备材料:

蒸馏水、温箱或水浴锅

操作步骤: (仅供参考)

1. 石蜡切片脱蜡至水, 冰冻切片直接入水。
2. 冰冻切片在丙酮-氯仿等量混合液内, 4°C 固定 2-5min。
3. 切片入 ALP 孵育液(阴性对照切片入 ALP 对照液), 置于 37°C 温箱。冰冻切片孵育 5-15min, 石蜡切片孵育 2-12h。流水洗 2min 后入蒸馏水。
4. 入 Co 溶液, 置于 37°C 温箱染色 5min。流水洗 5min 后入蒸馏水。
5. 上述过程中配制 ALP 硫化工作液, 即取试剂(C)用蒸馏水稀释即为 ALP 硫化工作液, 即配即用。切片入硫化工作液, 孵育 1-2min。流水洗 10min 后入蒸馏水。
6. 入氧化剂室温氧化 5-10min。蒸馏水换洗。
7. 入 Schiff 试剂, 置于温箱染色 10-15min。
8. 亚硫酸溶液: 蒸馏水按 1:10 的比例配制亚硫酸工作液, 清洗 2min, 入蒸馏水洗 5min。

9. 冰冻切片直接用甘油明胶封片，石蜡切片脱水。常规透明，中性树胶封片。

染色结果：

碱性磷酸酶活性部位	黑色
PAS 阳性物质	紫红色

阴性对照：(可选)

1. ALP 对照液为不含底物的孵育液。取相同的切片入 ALP 对照液，其余同上。结果为阴性。
2. (备选方案)切片进入 ALP 孵育液前，可先经碘液和 5% 硫代硫酸钠溶液各 3min，充分水洗后再进行孵育等步骤，可用此法作阴性对照。

注意事项：

1. 本染色液适用于冰冻切片、石蜡切片。染色过程中注意控制氧化剂的处理时间，否则容易褪色。
2. 碱性磷酸酶显色后，经氧化剂应特别小心，严格控制时间，否则褪色。
3. ALP 孵育液、ALP 硫化液易失效，最好分成小份储存，一经开启立即使用。
4. ALP 硫化液具有腐蚀性和刺激性气味，应小心操作。
5. 对冰冻切片染色时，应减少切片在室温暴露的时间。

Alkaline Phosphatase-PAS Stain Kit

Cat: G2450

Size: 6×50mL

Storage: 2-8°C, avoid light, valid for 6 months.

Kit Components

Reagent	6×50mL	Storage
Reagent(A): ALP Incubation Solution	50mL	2-8°C, avoid light
Reagent(B): Co Solution	50mL	RT, avoid light
Reagent(C): ALP Vulcanizing Solution	2×1mL	RT, avoid light
Reagent(D): Oxidant	50mL	2-8°C, avoid light
Reagent(E): Schiff Reagent	50mL	2-8°C, avoid light
Reagent(F): Sulphite Solution	50mL	RT, avoid light
Reagent(G): ALP Control Solution	10mL	2-8°C, avoid light

Introduction

Alkaline phosphatase (ALP or AKP) is a kind of phosphatase, which is widely distributed in mammalian tissues, and the optimal pH of its activity is 9.2-9.8. This enzyme is mainly found in the active sites of material exchange (cell membrane), such as the brush border of intestinal epithelium and renal proximal convoluted tubules, the static cilia of epididymal epithelium, the membrane of bile capillaries of liver, the endothelium of arterioles and capillaries, and also in the endoplasmic reticulum, golgi complex, swallowing vesicles, lysosomes of intestinal epithelium, neutrophils of neutrophils, and the membrane of smooth muscle.

In 1946, McManus first used PAS method to display mucin, which is often used to display glycogen and other polysaccharides. The oxidant can oxidize the 1,2-glycol group in sugars and related substances to make it dialdehyde. Aldehyde and Schiff reagent can combine to form a fuchsin compound to produce purplish red. Because oxidants can also oxidize other substances in cells, we should pay attention to the selection of concentration and oxidation time, so that the oxidation can be controlled not only to oxidize glycol group to aldehyde group, but also to avoid peroxidation.

ALP-PAS Stain kit can not only show the activity site of alkaline phosphatase and glycogen, but also distinguish them. Frozen section and paraffin section can be used.

Self Provided Materials

Distilled water, Incubator or Water bath

Protocol(for reference only)

1. For paraffin section, dewax in xylene and dehydrate in ethanol, then put into water. For frozen section, directly put into water.
2. Fix frozen section in the mixture of equal amount acetone and chloroform were for 2-5min at 4 °C.
3. Take the section into ALP Incubation Solution (take negative control section into ALP Control Solution) and place it in 37 °C incubator. The frozen section is incubated for 5-15min and paraffin section for 2-12h. Rinse with running water for 2min and then put into distilled water.
4. Add Co Solution and stain in 37 °C incubator for 5min. Rinse with running water for 5min and then put into distilled water.

5. In the above process, dilute Reagent (C) with distilled water to form ALP Vulcanizing Working Solution. It is ready to use. Take the section into ALP Vulcanizing Working Solution and incubate for 1-2min.
6. Rinse with running water for 10min and then put into distilled water.
7. Oxidize with oxidant at room temperature for 5-10min. Rinse with distilled water.
8. Place the section into Schiff Reagent and dye in the incubator for 10-15min.
9. Prepare Sulphite Working Solution according to the ratio of Sulphite Solution: distilled water is 1:10. Wash with running water for 2min and wash with distilled water for 5min .
10. For frozen section, directly seal with glycerin gelatin. For paraffin section, conventionally dehydrate in ethanol and transparent by xylene, then seal with resinene.

Result

Alkaline Phosphatase Active Site	Black
PAS Positive Substance	Purplish Red

Positive Control(optional):

1. ALP Control Solution is an incubation solution without substrate. Take the same section into ALP Control Solution, the rest follow the above steps. The result is negative.
2. (*Alternative*) Before the section enters into the ALP Incubation Solution, it can be rinsed by Iodine Solution and 5% Sodium Thiosulfate Solution for 3min respectively, and wash with water fully. Then it can be incubated and continue the rest steps. This method can be used as a negative control.

Note

1. The staining solution is suitable for frozen section and paraffin section. Pay attention to control the treatment time of oxidant in dyeing process, otherwise it is easy to fade.
2. After the color development of alkaline phosphatase, pay more attention after oxidation by oxidant, and control the time strictly, otherwise the color will fade.
3. ALP Incubation Solution and ALP Vulcanizing Solution are easy to lose effect, so it is better to store them in small parts and use them immediately once open.
4. ALP Vulcanizing Solution has corrosive and irritating smell, so operate carefully.
5. The exposure time of frozen section at room temperature should be reduced when staining frozen section.