碱性磷酸酶染色试剂盒(改良 Gomori 钙钴法)

货号： G1481
规格： 3×50mL
保存： 2-8℃, 避光保存, 有效期 6 个月。

产品组成：

名称	3×50mL	保存
试剂(A): ALP 孵育液	50mL	2-8℃, 避光
试剂(B): Co 溶液	50mL	室温, 避光
试剂(C): 硫化液	2×1mL	室温, 避光
试剂(D): ALP 对照液	10mL	2-8℃ ,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。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。

本试剂盒用金属沉淀法来显示碱性磷酸酶活性。此法以天然存在的β-甘油磷酸钠为底物，经酶水解释放出磷酸，立即被钙离子沉淀为磷酸钙，再次被置换为磷酸钴,最终被硫化液置换为黑色沉淀。

自备材料：

蒸馏水、 温箱或水浴锅

操作步骤：（*仅供参考*）

(一)石蜡切片染色

1. 石蜡切片脱蜡至蒸馏水。
2. 切片入 ALP 孵育液中，37℃孵育 2-12h。 流水洗 2min，入蒸馏水。
3. 入试剂 B 中，37℃孵育 5min。 流水洗 5min 后，入蒸馏水。
4. 在上述过程中，配制硫化工作液，即取试剂(C)用蒸馏水或者去离子水稀释 50 倍,即为硫化工作液，即配即用。切片入硫化工作液，孵育 1-2min。 流水洗 10min，入蒸馏水。
5. (*可选*)核固红复染细胞核，蒸馏水洗。
6. 石蜡切片常规脱水、透明，树胶封片。

(二)冰冻切片染色

1. 冰冻切片在丙酮-氯仿等量混合液内，4℃固定 2-5min。
2. 切片入 ALP 孵育液，37℃孵育 45-75min。流水洗 2min，入蒸馏水。
3. 入试剂 B 中，37℃孵育 5min。 流水洗 5min 后，入蒸馏水。
4. 在上述过程中，配制硫化工作液，即取试剂(C)用蒸馏水或者去离子水稀释 50 倍,即为硫化工作液，即配即用。切片入硫化工作液，孵育 1-2min。流水洗 10min，入蒸馏水。
5. (*可选*)核固红复染细胞核，蒸馏水洗。
6. 冰冻切片用甘油明胶封片。

染色结果：

酶所在阳性部位	黑色硫化钴沉淀
细胞核	红色（核固红复染）

阴性对照(*可选*):

1. 试剂(D)为不含底物的孵育液。取相同的切片入试剂(D)--ALP 对照液，而不是 ALP 孵育液，其余相同。阴性对照结果为阴性。
2. (备选方案)切片进入孵育液前，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. 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。
2. 硫化液具有腐蚀性，操作应小心。
3. 对冰冻切片染色时，应减少切片在室温暴露的时间。
4. 样本需新鲜，取材后应立即处理，否则会影响酶的活性。
5. 组织固定需在 4℃冰箱进行，时间不宜超过 24h，否则酶活性会减弱或消失。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Alkaline Phophatase Stain Kit (Modified Gomori Ca-CoS Method)

Cat: G1481
Size:3×50mL
Storage: 2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent	3×50mL	Storage
Reagent(A): ALP Incubation Solution	50mL	2-8℃, avoid light
Reagent(B): Co Solution	50mL	RT, avoid light
Reagent(C): Vulcanizing Solution	2×1mL	RT, avoid light
Reagent(D): ALP Contrast Solution	10mL	2-8℃, avoid light

Introduction

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

This kit uses metal precipitation method to display the alkaline phosphatase activity. Alkaline phosphatase hydrolyzes to release phosphoric acid using naturally occurring sodium beta-glycerophosphate as the substrate. Then the phosphoric acid is precipitated into calcium phosphate by calcium ion immediately, replaced with cobalt phosphate by Co Solution again, and finally replaced with black precipitation by Vulcanizing Solution.

Self Provided Materials

Distilled water, Incubator, Water bath

Protocol (*for reference only*)

For paraffin section staining

1. Dewax paraffin sections and rehydrate in graded alcohol.
2. Incubate the section in ALP Incubation Solution at 37℃ for 2-12 h.
3. Rinse with running water for 2mins and place it in distilled water.
4. Incubate the section in Co Solution at 37℃ for 5mins.
5. Rinse with running water for 5mins and place it in distilled water.
6. In the above-mentioned process, dilute Reagent (C) to 50 times with distilled water or deionized water to form the Vulcanizing Working Solution *(It is best to prepare it before use and it is not advisable to prepare in advance)*. Incubate the section in Vulcanizing Working Solution for 1-2mins.
7. Rinse with running water for 10mins and place it in distilled water.
8. *(Optional)* Re-dying with Nuclear Fast Red and wash with distilled water.
9. Absolute ethanol dehydration, transparent by xylene, seal with resinene.

For frozen section staining

1. Fix the frozen section in acetone-chloroform equal mixture at 4℃ for 2-5min .
2. Incubate the section in ALP Incubation Solution at 37℃ for 45-75min.
3. Rinse with running water for 2min and place it in distilled water.
4. Incubate the section in Co Solution at 37℃ for 5min.
5. Rinse with running water for 5mins and place it in distilled water.
6. In the above-mentioned process, dilute Reagent (C) to 50 times with distilled water or deionized water to form the Vulcanizing Working Solution *(It is best to prepare it before use and it is not advisable to prepare in advance)*. Incubate the section in Vulcanizing Working Solution for 1-2min.
7. Rinse with running water for 10min and place it in distilled water.
8. *(Optional)* Re-dying with Nuclear Fast Red and wash with distilled water.
9. Seal with Glycerol gelatin.

Result

Positive Site of Enzyme	Black Cobalt Sulfide Precipitation
Nucleus	Red(Re-dying with Nuclear Fast Red)

Negative Control(*optional*)

1. Reagent (D) is a substrate-free incubation solution. Take the same section into the Reagent (D) - ALP Contrast Solution instead of ALP Incubation Solution, and follow the steps. The negative control result is negative.
2. (*Alternative*)The section can wash in iodine solution and 5% sodium thiosulfate solution each for 3mins and wash with water fully before incubating in incubation solution. This method can use as a negative control.

Note

1. ALP Incubation Solution and ALP Vulcanizing Solution are easy to lose effect. It's better to store them in small fractions and use them as soon as open.
2. Vulcanizing Solution is corrosive and operate with caution.
3. When staining frozen sections, the exposure time of sections at room temperature should be reduced.
4. Samples should be fresh and treated immediately after sampling, otherwise the enzyme activity will be affected.
5. Tissue should be fixed in refrigerator at 4℃ no more than 24 h, otherwise the activity of enzyme will be weakened or disappeared.
6. For your safety and health, please wear experimental clothes and disposable gloves.