V02

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
将试剂 C 用蒸馏水或去离子水稀释 50 倍即为硫化工作液，现配现用。		
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 、1%氯化钙水溶液

操作步骤：（*仅供参考*）

(一)石蜡切片染色 （见注意事项 3）
1. 低熔点蜡包埋样本制备切片，常规脱蜡至蒸馏水。
2. 滴加试剂(A):ALP 孵育液覆盖切片 37℃孵育 2-12h。
3. 1%氯化钙处理 3 次，每次 1min，每次处理后倾去多余液体，不可大力冲洗。（见注意事项 4）
4. 滴加试剂(B): Co 溶液覆盖切片 37℃孵育 5min，蒸馏水冲洗 2min 后，浸入蒸馏水备用。
5. 取试剂(C): 硫化液用蒸馏水稀释 50 倍, 配制硫化工作液，即配即用。（见注意事项 2）
6. 滴加硫化工作液覆盖切片孵育 1-2min，目测变黑即可。蒸馏水浸洗 3 次，每次 3min。
7. (*可选*)核固红复染细胞核 3min，蒸馏水洗 1min。
8. 切片常规脱水、透明，中性树胶封片。

(二)冰冻切片染色 （见注意事项 3）
1. 冰冻切片取出复温后，滴加丙酮固定 2-5min。
2. 倾去多余固定液后滴加试剂(A):ALP 孵育液覆盖切片 37℃孵育 45-75min。
3. 1%氯化钙处理 3 次，每次 1min，每次处理后倾去多余液体，不可大力冲洗。（见注意事项 4）
4. 滴加试剂(B): Co 溶液覆盖切片 37℃孵育 5min，蒸馏水冲洗 2min 后，浸入蒸馏水备用。
5. 取试剂(C): 硫化液用蒸馏水稀释 50 倍, 配制硫化工作液，即配即用。（见注意事项 2）
6. 滴加硫化工作液覆盖切片孵育 1-2min，目测变黑即可。蒸馏水浸洗 3 次，每次 3min。
7. (*可选*)核固红复染细胞核 3min，蒸馏水洗 1min。
8. 切片用甘油明胶封片，或常规脱水、透明后中性树胶封片。

染色结果：

阳性部位	黑色沉淀
细胞核	红色（核固红复染）

阴性对照(*可选*)：

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

1. 试剂阴性：试剂(D)为不含底物的孵育液。取相邻的切片滴加试剂(D): ALP 对照液替代剂(A):ALP 孵育液进行孵育，其染色结果为试剂阴性。
2. 酶活阴性：切片染色前经碘液和 5%硫代硫酸钠溶液各 3min 灭活碱性磷酸酶，充分水洗后再进行孵育等步骤，其染色结果为酶活阴性。

注意事项：

1. 试剂(A):ALP 孵育液为无菌溶液，建议收到试剂盒后于超净台分装小份储存，一经开启立即使用。
2. 硫化液有特殊气味且具有腐蚀性，使用和稀释操作应在通风橱内小心进行。
3. 碱性磷酸酶较脆弱，样本取材后应立即处理。固定推荐在 2-8℃冰箱进行，时间不宜超过 24h。包埋温度建议不超过 60℃，时间不超过 3 小时，避免失活导致无阳性。
4. 由于形成的磷酸钙微溶于水，使用蒸馏水清洗可能会洗脱磷酸钙导致后续染色无阳性或弱阳性，1%氯化钙轻柔清洗会补充钙离子保证磷酸钙稳定。
5. 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)

V02

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
Dilute Reagent(C) 50 times with distilled water or deionized water to obtain the Vulcanization Working Solution, which is prepared and used immediately.		
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, 1% calcium chloride aqueous solution

Protocol (*for reference only*)

For paraffin section*(See Note 3)*

1. Dewax paraffin sections and rehydrate in graded alcohol to distilled water.
2. Cover the section with Reagent(A): ALP Incubation Solution and incubate at 37℃ for 2-12 h.
3. Treat with 1% calcium chloride three times, each time for 1 minute. After each treatment, pour out excess liquid and do not rinse vigorously. (See Note 4)
4. Cover the slices with Reagent (B): Co solution and incubate at 37 ℃ for 5 min.
5. Rinse with running water for 5min and place it in distilled water.
6. Dilute reagent (C):Vulcanizing Solution 50 times with distilled water to prepare the Vulcanization Working Solution, which is ready to use. (See Note 2)
7. Cover the section with Vulcanizing Working Solution and incubate for 1-2min till it turn black.
8. Rinse with distilled water for 3 times, each time for 3 min.
9. *(Optional)* Re-dying with Nuclear Fast Red for 3min and wash with distilled water for 1min.
10. Absolute ethanol dehydration, transparent by xylene, seal with resinene.

For frozen section*(See Note 3)*

1. Fix the frozen section by acetone for 2-5min .
2. Cover the section with Reagent(A): ALP Incubation Solution and incubate at 37℃ for 45-75 h.
3. Treat with 1% calcium chloride three times, each time for 1 minute. After each treatment, pour out excess liquid and do not rinse vigorously. (See Note 4)
4. Cover the slices with Reagent (B): Co Solution and incubate at 37 ℃ for 5 min.
5. Rinse with running water for 5min and place it in distilled water.
6. Dilute reagent (C):Vulcanizing Solution 50 times with distilled water to prepare the Vulcanization Working Solution, which is ready to use. (See Note 2)
7. Cover the section with Vulcanizing Working Solution and incubate for 1-2min till it turn black.
8. Rinse with distilled water for 3 times, each time for 3 min.

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9. (Optional) Re-dying with Nuclear Fast Red for 3min and wash with distilled water for 1min.
10. Seal with Glycerol gelatin or usually dehydrate, transparent by xylene, seal with resinene.

Result

Positive Site	Black Precipitation
Nucleus	Red(By Nuclear Fast Red)

Negative Control(*optional*)

1. Reagent negative: Reagent (D) is a substrate free incubation solution. Take adjacent slices and add Reagent (D): ALP Contrast Solution substitute Reagent(A):ALP Incubation Solution for incubation. The staining result is negative for the reagent.
2. Negative enzyme activity: Before staining the sections, alkaline phosphatase was inactivated in iodine solution and 5% sodium thiosulfate solution for 3 min each. After thorough washing with water, incubation and other steps were carried out. The staining result was negative for enzyme activity.

Note

1. Reagent (A): ALP Incubation Solution is a sterile solution. It is recommended to receive the reagent kit and store it in small portions on the ultra clean table. Once opened, it should be used immediately.
2. Reagent(C): Vulcanizing Solution has a special odor and is corrosive. Use and dilution operations should be carried out carefully in a fume hood.
3. Alkaline phosphatase is relatively fragile, and samples should be processed immediately after sampling. It is recommended to use a refrigerator at 2-8 ℃ for a fixed period of time, which should not exceed 24 hours. The recommended embedding temperature should not exceed 60 ℃ and the time should not exceed 3 hours to avoid inactivation leading to no positive results.
4. Due to the formation of calcium phosphate that is slightly soluble in water, washing with distilled water may wash away the calcium phosphate, resulting in subsequent staining without positive or weak positive results. Gentle cleaning with 1% calcium chloride will replenish calcium ions to ensure the stability of calcium phosphate.
5. For your safety and health, please wear laboratory clothes and disposable gloves when operating.