V02

酸性磷酸酶染色试剂盒(金属沉淀法)

货号：G1490
规格：2×50mL
保存：2-8℃，避光保存，有效期 6 个月。

产品组成：

名称	2×50mL	保存
试剂(A): ACP 孵育液	50mL	2-8℃，避光
试剂(B): ACP 硫化液	2×1mL	室温，避光
试剂(C): ACP 对照液	10mL	2-8℃，避光

产品介绍：

酸性磷酸酶（Acid phosphatase，ACP）分布极广泛，遍布各种组织，主要存在于细胞的溶酶体内，所以常作为溶酶体标志酶。溶酶体外的酸性磷酸酶存在于内质网和胞质内。各种动物中的酸性磷酸酶各有不同，酸性磷酸酶的适宜 pH 为 4.5-5.5。

酸性磷酸酶染色试剂盒以β-甘油磷酸钠为底物，在酸性 pH 下被酸性磷酸酶水解释放出磷酸盐，遇到铅离子则生成磷酸铅沉淀，再被 S^{2+}置换，最终生成硫化铅棕黑色沉淀。酸性磷酸酶的一般抑制剂为氟化物、磷酸根离子。对某些酸性磷酸酶来讲，Cu^{2+}、酒石酸根离子和四氯化碳以及醛类也都是抑制剂，Mn^{2+}为该酶的激活剂。冰冻切片和石蜡切片均可，但多用冰冻切片。临床上，该染色法对前列腺癌和其他脏器的转移性前列腺癌呈强阳性反应，霍奇金淋巴瘤、胃癌、肺癌、乳腺癌、舌表皮性癌、多核巨细胞瘤的瘤细胞质也呈强阳性反应，对 Ewing 肉瘤、成骨肉瘤等呈阴性反应。

自备材料：

蒸馏水、恒温箱、封片剂

操作步骤：(*仅供参考*)

（一）冰冻切片染色

1. 冰冻切片至蒸馏水。
2. 切片放入湿盒，滴加 ACP 孵育液至切片上，置于 37℃温箱恒温染色 15-60min。
3. 滴加预热至 37℃的蒸馏水清洗切片 2 次，每次 1min，以去除未被吸附的铅。
4. 在上述过程中配制 ALP 硫化工作液，即取试剂（B）用蒸馏水稀释 50 倍，即为 ALP 硫化工作液，即配即用。切片上滴加 ALP 硫化工作液，孵育 1-2min。
5. 蒸馏水洗 3-5min。
6. （*可选*）核固红复染细胞核，蒸馏水洗。甘油明胶封片。

（二）石蜡切片染色

1. 石蜡切片脱蜡至蒸馏水。
2. 滴加 ACP 孵育液，置于 37℃温箱恒温浸染 4-12h，可以延长至 24h。
3. 滴加预热至 37℃的蒸馏水清洗切片 2 次，每次 1min，以去除未被吸附的铅。
4. 在上述过程中配制 ALP 硫化工作液，即取试剂（B）用蒸馏水稀释 50 倍，即为 ALP 硫化工作液，即配即用。切片上滴加 ALP 硫化工作液，孵育 1-2min。
5. 蒸馏水洗 3-5min。
6. （*可选*）核固红复染细胞核，蒸馏水洗。甘油明胶封片。

染色结果：

酶活性部位	黑色
细胞核	根据复染液不同而不同

阴性对照：（*可选*）

将切片置于试剂（C）：ACP 对照液中，室温 1-2h 孵育，其余步骤相同，结果为阴性。

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注意事项：

1. ACP 孵育液、ACP 硫化液易失效，最好分成小份储存。ACP 硫化液具有腐蚀性。
2. 对冰冻切片染色时，应减少切片在室温暴露的时间。
3. 样本需新鲜，取材后应立即处理，否则会影响酶的活性。
4. 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。
5. 组织在石蜡包埋时，温度不宜高于 56℃。应使用熔点为 52-54℃的石蜡进行浸蜡，浸蜡时间要短，否则酶活性会减弱或消失。
6. 不纯的二甲苯会分解黑色沉淀，宜选用 AR 级以上的二甲苯。
7. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

V02

Acid Phophatase Stain Kit(Metal Precipitation Method)

Cat: G1490
Size: 2×50mL
Storage: 2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent	2×50mL	Storage
Reagent A : ACP Incubation Buffer	50mL	2-8℃, avoid light
Reagent B :ACP Vulcanizing Solution	2×1mL	RT, avoid light
Reagent C : ACP Control Solution	10mL	2-8℃, avoid light

Introduction

Acid phosphatase (ACP) is widely distributed in various tissues, mainly in the lysosome, so it is often used as a lysosome marker enzyme. The acid phosphatase outside of lysozyme exists in endoplasmic reticulum and cytoplasm. Acidic phosphatase is different in all kinds of animals. The optimum pH for its activity is 4.5-5.5.

The Acid Phophatase Stain Kit(Metal Precipitation Method) is used as a substrate, which is hydrolyzed by acid phosphatase at an acidic pH to release phosphate. When it encounters lead ions, it forms a lead phosphate precipitate, which is then replaced by S2+, ultimately resulting in a brown black sulfide precipitate. The general inhibitors of acid phosphatase are fluoride and phosphate ions. For certain acid phosphatase enzymes, Cu2+, tartrate ions, carbon tetrachloride, and aldehydes are also inhibitors, and Mn2+is the activator of the enzyme. The Acid Phophatase Stain Kit(Metal Precipitation Method) is suitable for frozen section and paraffin section, but frozen section is more recommended. Clinically, the staining method showed strong positive reaction to prostate cancer and metastatic prostate cancer of other organs, and also showed strong positive reaction to the tumor cytoplasm of Hodgkin's lymphoma, gastric cancer, lung cancer, breast cancer, tongue epidermal cancer, multinuclear giant cell tumor, and negative reaction to Ewing's sarcoma, osteosarcoma, etc.

Self Provided Materials

Distilled H_2O, Incubator, Sealing agent

Protocol(*for reference only*)

Frozen Section Stain

1. Restore the frozen sections to room temperature by distilled water for 2 min.
2. Add ACP Incubation Buffer to the section in the wet box and place it in a 37℃ incubator for 15-60 min.
3. To remove lead which has not been specially adsorbed,wash in distilled water at 37℃ for twice and 1min each time.
4. Dilute Reagent B with water 50 times to form ACP Vulcanizing Working Solution in the above process.It is ready for use.Incubate the sections with ACP Vulcanizing Working Solution for 1-2 min.
5. Rinse with distilled water for 3-5 min.
6. (*Optional*)Re-dyeing the nucleus with Nuclear Fast Red and wash with distilled water. Glycerol gelatin seals.

Paraffin Section Stain

1. Dewax paraffin sections and rehydrate in graded alcohol.
2. Soak the section into ACP Incubation Buffer and place it in a 37℃ incubator for 4-12h. According to the results of dyeing, it could be prolonged to 24 h.
3. To remove lead which has not been specially adsorb,wash in distilled water at 37℃ for twice and 1 min each time.
4. Dilute Reagent B with water 50 times to form ACP Vulcanizing Working Solution in the above process. It is ready for use. Incubate the sections with ACP Vulcanizing Working Solution for 1-2 min.
5. Rinse with distilled water for 3-5 min.
6. (*Optional*)Re-dyeing the nucleus with Nuclear Fast Red and wash with distilled water. Glycerol gelatin seals.

Result

Positive Site	Black

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Nucleus	According to the re-dyeing solution.

Negative Control（*Optional*）

Incubate the sections at room temperature for 1-2 h in ACP Control Solution.The other steps are the same, and the result was negative.

Note

1. ACP Incubation Buffer and ACP Vulcanizing Solution are easy to lose effect and should be divided into the vials. ACP Vulcanizing Solution has corrosive and irritating odor and should be carefully operated.
2. When staining frozen sections, the exposure time of sections at room temperature should be reduced.
3. Samples should be fresh and treated immediately after sampling, otherwise the enzyme activity will be affected.
4. Fixed tissue should be carried out in a refrigerator at 4℃ for no more than 24h. Otherwise, the enzyme activity will weaken or disappear.
5. Use the paraffin with melting point of 52-54℃ for wax and the time of wax should be short, otherwise the enzyme activity will weaken or disappear.
6. Impure xylene will decompose black precipitation. It is better to choose xylene above AR grade.
7. For your safety and health, please wear experimental clothes and disposable gloves.