葡萄糖-6-磷酸酶染色试剂盒(金属沉淀法)

货号： G1500
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): G-6-Pase 孵育液	50mL	2-8℃, 避光
试剂(B): ALP 硫化溶液	2×1mL	室温, 避光
试剂(C): 中性福尔马林	50mL	2-8℃, 避光
试剂(D): G-6-Pase 对照液	10mL	2-8℃, 避光

产品介绍：

葡萄糖-6-磷酸酶(glucose-6 –phosphatase，G-6-Pase)是一种多存在于哺乳类动物肝、肾、肠等组织的膜结合酶，G-6-Pase 和微体紧密结合，定位于内质网，是内质网的主要标志酶，能把葡萄糖-6-磷酸水解成葡萄糖和磷酸。G-6-Pase 对维持血糖浓度的相对恒定有至关重要的作用，是糖代谢的关键酶。当血糖降低时，G-6-Pase 促进肝糖原转变为血糖。当缺乏 G-6-Pase 时，会引起糖原分解障碍，使糖原积累在肝、肾、心脏等部位，导致肝糖原储积病。G-6-Pase 最适 pH 为 6.5，在 pH6.0-8.0 亦可，pH8.0 最稳定，pH5.0 易变性。组织化学反应多用 pH6.5-6.7。

葡萄糖-6-磷酸酶染色试剂盒(金属沉淀法)采用重金属捕捉剂和磷酸结合显示酶的活性。该酶对固定很敏感，组织经 80%乙酸溶液固定后用石蜡包埋，G-6-Pase 完全被抑制。需用新鲜组织低温恒冷切片，经甲醛短时固定酶即失活，可短时低温丙酮固定，但一般不固定。

操作步骤：(*仅供参考*)

1. 冰冻切片入蒸馏水清洗。
2. 入 G-6-Pase 孵育液 37℃孵育 20min。
3. 自来水冲洗后，蒸馏水冲洗。
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。切片入硫化工作液，孵育 1min。自来水水洗。
5. (*可选*)入中性福尔马林，固定 2min。入蒸馏水水洗 2 次。
6. 甘油明胶封片。

染色结果：

G6Pase 活性处	棕色沉淀

阴性对照(*可选*)： 将切片置入试剂(D)- G-6-Pase 对照液中，其余步骤相同，结果为阴性。

注意事项：

1. ALP 硫化液易失效，最好分成小份储存，一经开启立即使用。
2. ALP 硫化液具有腐蚀性和刺激性气味，应小心操作。
3. 对冰冻切片染色时，应减少切片在室温暴露的时间。
4. 本染色液适用于冰冻切片，一般不固定，也可染色后固定，固定步骤非必须步骤。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Glucose-6-Phosphatase Stain Kit(Metal Precipitation Method)

Cat: G1500
Size: 3×50mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent	3×50mL	Storage
Reagent(A): G-6-Pase Incubation Solution	50mL	2-8℃, avoid light
Reagent(B): ALP Vulcanizing Solution	2×1mL	RT, avoid light
Reagent(C): Neutral Formalin	50mL	2-8℃, avoid light
Reagent(D): G-6-Pase Control Solution	10mL	2-8℃, avoid light

Introduction

Glucose-6-phosphatase (G-6-Pase) is a membrane-binding enzyme that exists in mammalian liver, kidney, intestine and other tissues. G-6-Pase is closely bound to microbodies and located in the endoplasmic reticulum. It is the main marker enzyme of endoplasmic reticulum. It can hydrolyze glucose-6-phosphate into glucose and phosphoric acid.G-6-Pase is a key enzyme in glycometabolism, which plays an important role in maintaining the relative constant blood sugar concentration. When blood sugar is lowered, G-6-Pase promotes glycogen conversion from liver to blood sugar. When lacking G-6-Pase, it will cause glycogen decomposition disorder and accumulate glycogen in liver, kidney, heart and other parts, leading to glycogen storage disease. The optimum pH of G-6-Pase is 6.5, which is also acceptable at pH 6.0-8.0. The most stable pH of G-6-Pase is 8.0, which is changeable at pH 5.0. PH 6.5-6.7 is commonly used in histochemical reactions.

Glucose-6-Phosphatase Stain Kit(Metal Precipitation Method) used heavy metal traps and phosphoric acid binding to show the activity of the enzyme. The enzyme is sensitive to immobilization and G-6-Pase is completely inhibited after paraffin-embedded tissues are immobilized in 80% acetic acid solution. Enzyme also become inactivated after short-term formaldehyde immobilization. It is recommended to use cryostatic section of fresh tissue, which can be fixed by acetone at low temperature for a short time, but not in general.

Protocol (*for reference only*)

1. Restore the frozen sections to room temperature by distilled water for 2mins.
2. Incubate the section in G-6-Pase Incubation Solution at 37℃ for 20min.
3. To remove lead which has not been specially adsorbed, wash in distilled water for 2 times and 1 min each time.
4. Dilute Reagent B with water 50 times to form ALP Vulcanizing Working Solution in the above process. It is ready for use. Incubate the sections with ALP vulcanizing working solution for 1 min.
5. Rinse with distilled water for 3-5min twice.
6. *(Optional)* Fix in Neutral Formalin for 2min.
7. Rinse with distilled water for 3-5min twice.
8. Glycerol gelatin seals.

Result

G-6-Pase Positive Site	Brown precipitation

Negative control (*Optional*): Immerse the section into Reagent (D) G-6-Pase Control Solution, the other steps are the same, and the result is negative.

Note

1. ALP Vulcanizing Solution is easy to lose effect and should be divided into the vials. ALP 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. Samples should be fresh and treated immediately after sampling, otherwise the enzyme activity will be affected. Fixed tissue should be carried out in a refrigerator at 4℃ for no more than 24 h. Otherwise, the enzyme activity will weaken or disappear.
3. This staining solution is suitable for frozen sections. It is not fixed or fixed after dyeing in general. Fixing steps are not necessary.
4. For your safety and health, please wear experimental clothes and disposable gloves.