V02

糖原 D-PAS 染色试剂盒（淀粉酶消化法）

货号：G1282
规格：5×50mL/5×100mL
保存：2-8℃，避光保存，有效期 6 个月。

产品组成：

名称	5×50mL	5×100mL	保存
试剂（A）：淀粉酶溶液	50mL	100mL	2-8℃
试剂（B）：PAS 氧化剂	50mL	100mL	2-8℃，避光
试剂（C）：Schiff 染色液	50mL	100mL	2-8℃，避光
试剂（D）：Mayer 苏木素染色液	50mL	100mL	2-8℃，避光
试剂（E）：酸性分化液	50mL	100mL	室温

产品介绍：

糖原染色是病理学中常规的染色方法之一，McManus 在 1946 年最先使用 PAS 技术显示黏蛋白，该法常用来显示糖原和其他多糖，该染色液不仅能够显示糖原，还能显示中性黏液性物质和某些酸性物质，以及软骨、垂体、霉菌、真菌、色素、淀粉样物质、基底膜等。

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氧化剂浓度和氧化时间，使氧化控制在即能把乙二醇基氧化成醛基，又不至于过氧化，这是很关键的步骤。

PAS 技术是唯一可检测不同种类的黏液物质（如糖原、黏蛋白和糖蛋白）的方法，但 PAS 技术却不能区别黏蛋白和糖原。若要准确鉴别黏液物质（如黏蛋白和糖原），需加入糖原消化步骤。大多数情况下可用α-淀粉酶或麦芽淀粉酶来催化糖原的糖苷键水解。形成水溶性的双糖-麦芽糖，在应用 PAS 技术之前将糖原从组织切片上除去。人类的唾液被认为是消化糖原的一种有效手段，但是出于安全以及缺乏标准唾液的考虑，不主张应用唾液。

糖原 D-PAS 染色试剂盒的特点在于糖原 PAS 染色之前经淀粉酶处理，糖原消化时需要两张相同的切片，脱蜡后一张切片用含有淀粉酶的适当缓冲液处理，另一张仅用缓冲液处理。然后两张切片均用 PAS 法染色，消化后染色消失表明存在糖原。

操作步骤：（*仅供参考*）

1. 两张相同切片，二甲苯脱蜡，梯度乙醇入水。
2. 一张切片滴加淀粉酶溶液室温处理 20-60min 作阴性对照。另一张浸水保持湿润即可，无需额外处理。
3. 流水冲洗两张切片各 2-5min。
4. 滴加氧化剂，室温放置 5-8min，一般不宜超过 10min。蒸馏水浸洗 2 次，每次 30s。
5. 滴加 Schiff 染色液，置于室温阴暗处，浸染 10-20min。自来水冲洗 10min。
6. 滴加 Mayer 苏木素染色液中，染细胞核 1-2min。
7. (可选)滴加酸性分化液分化 2-5s。
8. 自来水冲洗 10-15min 使其返蓝。
9. 常规乙醇脱水，二甲苯透明，中性树胶封固。

染色结果：

糖原、中性、唾液黏蛋白	红紫色
各种糖蛋白	红紫色
细胞核	蓝色
未处理的切片，糖原呈亮红色或红紫色；淀粉酶处理的切片，糖原阴性。	

注意事项：

1. 切片脱蜡应尽量干净，否则影响染色效果。

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2. 需使用一张阳性对照片验证酶的活性。
3. 氧化剂氧化时间不宜过久，氧化时的温度以 18-22℃最佳。
4. 试剂 A、试剂 B、试剂 C 应置于 4℃密闭保存，使用时避免接触过多的阳光和空气。使用前，最好提前取出恢复到室温，避光暗处使用。
5. 酸性分化液应经常更换新液，其分化时间应该依据切片厚薄、组织的类别和酸性分化液的新旧而定，另外分化后自来水冲洗时间应该足够。
6. 在氧化剂和 Schiff 染色液中作用时间非常重要，该依据切片厚薄、组织的类别等决定。
7. 冷冻切片染色时间尽量要短。
8. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

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Periodic Acid Schiff Diastase (D-PAS) Stain Kit

Cat: G1282
Size:5×50mL/5×100mL
Storage: 2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent	5×50mL	5×100mL	Storage
Reagent (A):α - Diastase Solution	50mL	100mL	2-8℃
Reagent (B):PAS Oxidant	50mL	100mL	2-8℃, avoid light
Reagent (C):Schiff Reagent	50mL	100mL	2-8℃, avoid light
Reagent (D):Mayer Hematoxylin Solution	50mL	100mL	2-8℃, avoid light
Reagent (E):Acidic Differentiation Solution	50mL	100mL	RT

Introduction

Glycogen staining is one of the conventional staining methods in pathology. McManus first used PAS technology to display mucin in 1946. This method is often used to display glycogen and other polysaccharides. The staining solution can not only display glycogen, but also show neutral mucilaginous substances and some acidic substances, as well as cartilage, pituitary, mould, fungus, pigment, amyloid substance, basement membrane, etc.

The oxidant can oxidize 1,2-glycol group in sugars and related substances to make it into dialdehyde. Aldehyde and Schiff Reagent can combine to form a purplish-red fuchsin complex. As oxidants can also oxidize other substances in cells, we should pay attention to the concentration and oxidation time of oxidants when using, so that the oxidation control can oxidize glycol group to aldehyde group without peroxide, which is a key step.

The characteristic of Periodic Acid Schiff Diastase (D-PAS) Stain Kit is that before glycogen PAS staining, it is treated by diastase. When glycogen is digested, prepare two identical sections. After dewaxing, one section is treated with appropriate buffer containing diastase, and the other one is treated only with buffer. Then the two sections are stained with PAS method, and the disappearance of staining after digestion indicated the presence of glycogen.

Protocol (*for reference only*)

1. Prepare two identical sections and dewax to distilled water.
2. One slice was treated with amylase solution at room temperature for 20-60 min as a negative control. The other sheet can be soaked and kept wet without additional treatment.
3. Rinse the two sections in tap water respectively for 2-5min.
4. Add Oxidant to the sections and incubate for 5-8min at room temperature, generally no more than 10min. Rinse in tap water once and then rinse in distilled water twice.
5. Place the sections into Schiff Reagent and incubate in dark for 10-20min. Rinse in distilled water for 10min.
6. Stain with Mayer Hematoxylin Solution for 1-2min.
7. (optional) differentiate byAcidic Differentiation Solution for 2-5s. Rinse in tap water for 10-15min to blue.
8. Conventional dehydration by series of ethanol. Dehydrate, transparent by xylene and seal with resinene.

Result

Glycogen, Neutral and Salivary Mucin	Red to Purple
Various Glycoprotein	Red to Purple
Nucleus	Blue
The section untreated, glycogen is bright red or red purple; The section treated by Diastase Solution, glycogen shows negative.	

Note

1. Section dewaxing should be as clean as possible, otherwise it will affect the dyeing effect.
2. A positive control section is required to verify the enzyme activity.
3. The oxidation time of Oxidant should not be too long, and the best temperature is 18-22 ℃.
4. Reagent A, B and C should be kept in 4 ℃ airtight storage, and avoid too much sunlight and air during use. Before use, it is better to take it out in advance and restorm room temperature, and use it in dark.

5. Acid Differentiation Solution should be replaced frequently, and the differentiation time should be determined according to the thickness of section, the type of tissue and the old and new of Acid Differentiation Solution. In addition, the washing time of tap water after differentiation should be enough.
6. The time of action in Oxidant and Schiff Reagent is very important, which depends on the thickness of section and the type of tissue.
7. The staining time of frozen section should be as short as possible.
8. For your safety and health, please wear experimental clothes and disposable gloves.

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