福尔根核酸染色试剂盒

货号： G3070
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): Schiff 染色液		50mL	2-8℃，避光
试剂(B): SO2 水	试剂(B1)：弱酸溶液	50mL	室温，避光
	试剂(B2)：亚硫酸盐溶液	50mL	室温，避光

自备材料：

蒸馏水、系列乙醇、恒温箱

产品介绍：

脱氧核糖核酸(DNA)染色方法有福尔根法、甲基绿-派洛宁法、吖啶橙荧光法等，其中最经典的是 Feulgen 法，该法是一种经典的酶组织化学法。

福尔根核酸染色试剂盒原理在于 DNA 经温和的弱酸(例如盐酸)水解后，嘌呤碱与脱氧核糖间的糖苷键被打开，并且使脱氧核糖与磷酸间的磷酯键断开，在脱氧核糖的一端形成游离的醛基。醛基在原位与 Schiff 试剂结合，形成紫红色化合物，使细胞内含有 DNA 的部位呈紫红色。紫红色的产生是因为反应产物的分子内有醌基(醌基是一个具有颜色的发色团，所以凡含有 DNA 的部位就呈紫红色。该水解作用不影响核糖-嘌呤结合键，因此 RNA 用此法处理后则分解，所以该法不适用于证明 RNA。

操作步骤：(*仅供参考*)

（一）石蜡切片染色

1. 组织固定：Carnoy 固定石蜡切片较好，10%福尔马林亦可，不宜采用 Bouin 固定液。
2. 配制弱酸工作液：按弱酸溶液:蒸馏水=1:4 配制，即取 1 份弱酸溶液、4 份蒸馏水，充分混合，即获得弱酸工作液。
3. 石蜡切片脱蜡至蒸馏水。
4. 入弱酸工作液，室温浸洗一下。
5. 切片入预热至 60℃的弱酸工作液，孵育 8min。
6. 切片入室温的弱酸工作液中冲洗 1min。
7. 蒸馏水冲洗。
8. 切片入 Schiff 染色液，室温避光染色 30~60min。
9. 在上述染色过程中，配制 SO2 水工作液。按弱酸溶液:亚硫酸盐溶液:蒸馏水=1:5:94 配制，即取弱酸溶液 1 份、亚硫酸盐溶液 5 份、蒸馏水 94 份，充分混合，即配即用。
10. 用新鲜配制的 SO2 水工作液洗切片 3 次，每次 90s。
11. 蒸馏水中洗净。经系列乙醇脱水。二甲苯透明并封片。

（二）冰冻切片染色

1. 冰冻切片预处理：取 1 份乙酸、3 份无水乙醇混合即为固定液，固定 10min。
2. 由无水乙醇脱水--逐级下行—蒸馏水。
3. 配制弱酸工作液：按弱酸溶液:蒸馏水=1:4 配制，即取 1 份弱酸溶液、4 份蒸馏水，充分混合，即获得弱酸工作液。
4. 余下步骤同上述石蜡切片染色。

染色结果：

细胞核内DNA	红紫色

阴性对照：

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1. 将同样切片经上述步骤，只有步骤5 改为入室温弱酸工作液，孵育15min。
2. 结果为细胞核DNA 阴性。

注意事项：

1. 水解时间很重要，并且应使用恰当的固定时间。不同的固定液水解时间不一样。

固定液	水解时间(min)
Carnoy 固定液	8min
Helly 固定液	8min
Susa 固定液	18min
福尔马林	8min
Zenker液	5min

2. 注意Schiff 染色液的试剂状态，若变浅粉红亦可考虑使用，颜色变红则弃用。
3. 去除切片上多余Schiff染色液的方法以SO2水洗为好。
4. 建议进行阴性对照试验。

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Feulgen Nucleus Stain Kit

Cat: G3070
Size: 3×50mL
Storage: 2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		3×50mL	Storage
Reagent(A): Schiff Reagent		50mL	2-8℃, avoid light
Reagent(B): SO2 Solution	B1: Hydrochloric Acid Solution	50mL	RT, avoid light
	B2: Sodium Disulfite Solution	50mL	RT, avoid light

Self Provided Materials

Distilled water, Series of ethanol, Constant incubator.

Introduction

The methods of DNA staining include Feulgen method, Methyl Green-Pyronin method, Acridine Orange Fluorescence method and so on. The most classical one is Feulgen method, which is a classical enzyme histochemistry method.

The principle of this kit is that after DNA is hydrolyzed by mild weak acid (such as hydrochloric acid), the glycosidic bond between purine base and deoxyribose is opened, and the phosphoester bond between deoxyribose and phosphoric acid is broken, forming free aldehyde group at one end of deoxyribose. The aldehyde group combines with Schiff Reagent in situ to form a purplish red compound, which makes the part containing DNA in the cell show purplish red. The reason of purplish red is that there is a quinone group in the molecule of the reaction product (the quinone group is a chromophore with color, so the part containing DNA is purplish red). This hydrolysis does not affect the ribose-purine binding bond, so RNA will decompose after being treated with this method, so this method is not applicable to prove RNA.

Protocols(*for reference only*)

Paraffin section staining

1. Tissue fixation: for paraffin section, fixing in Carnoy is better, 10% formalin can also be used, but it is not suitable to use Bouin fixative.
2. Preparation of Hydrochloric Acid Working Solution: mix 1 part of Hydrochloric Acid Solution and 4 parts of distilled water to form Hydrochloric Acid Working Solution.
3. Dewax to distilled water.
4. Wash in Hydrochloric Acid Working Solution at room temperature.
5. Incubate in the Hydrochloric Acid Working Solution preheated to 60 ℃ for 8 min.
6. Wash in Hydrochloric Acid Working Solution at room temperature for 1 min.
7. Rinse with distilled water.
8. Put the section into Schiff Reagent and stain avoiding light at room temperature for 30-60 min.
9. In the above dyeing process, prepare SO2 Solution: take 1 part of Hydrochloric Acid Solution, 5 parts of Sodium Disulfite Solution and 94 parts of distilled water, then mix fully to form SO2 Solution. It is ready to use.
10. Wash the section in fresh SO2 Solution for 3 times, each time for 90s.
11. Wash in distilled water. Dehydrate in series of ethanol, transparent by xylene and seal.

Frozen section staining

1. Frozen section pretreatment: mix 1 part of acetic acid and 3 parts of anhydrous ethanol as the fixative and fix the section in fixative for 10mins.
2. Dehydration by absolute ethanol - step by step down - distilled water.
3. Prepare Hydrochloric Acid Working Solution: mix 1 part of Hydrochloric Acid Solution and 4 parts of distilled water to form Hydrochloric Acid Working Solution.
4. The remaining steps are the same as the above paraffin section staining.

Result

DNA in the nucleus	Red Purple

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Negative Control

1. Take the same section and operate according to the above steps(only update the step 5 that "Incubate in the Hydrochloric Acid Working Solution for 15 min").
2. The result shows that DNA in the nucleus is negative.

Note

1. Hydrolysis time is important and should fix for an appropriate time. The hydrolysis time of different fixatives is different.

Fixative	Hydrolysis Time(min)
Carnoy Fixative	8min
Helly Fixative	8min
Susa Fixative	18min
Formalin	8min
Zenker Fixative	5min

2. Pay attention to the color of Schiff Regent. If it turns light pink, can also use. If it turns red, discard it.
3. The best way to remove the remaining Schiff Reagent is washing in SO2 Solution.
4. Take a negative control test.

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