V02

SO_2 水溶液

货号：G3920
规格：2×100mL
保存：室温，避光保存，有效期 1 年。

产品组成：

名称	2×100mL	保存
试剂(A)：亚硫酸盐溶液	100mL	室温，避光
试剂(B)：弱酸溶液	100mL	室温
临用前 1:1 等量混合即为 SO_2 水工作液，建议现配现用。		

产品介绍：

Feulgen stain 的原理是 DNA 经弱酸(例如盐酸)水解后，嘌呤碱与脱氧核糖间的糖苷键被打开，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 染色液反应，形成紫红色化合物，使细胞内含有 DNA 的部位呈紫红色。

染色后用 SO_2 水溶液洗片，目的是洗去多余的非特异性色素及扩散的染料，使染色结果更加清晰、背景更加洁净。

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. 石蜡切片脱蜡至蒸馏水。
3. 切片入室温弱酸工作液（**自备**）浸洗后入预热至 60℃的弱酸工作液（**自备**），浸染孵育 8min。
4. 切片直接滴加 Schiff 染色液，室温避光染色 30~60min 至切片呈紫红色。
5. 在上述染色过程中，配制 SO_2 水工作液。
6. 使用新鲜配制的 SO_2 水工作液洗切片 3 次，每次 90s。
7. 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：使用 Carnoy 固定液（**自备**）固定 10min。
2. 由无水乙醇逐级下行至蒸馏水。
3. 余下步骤同上述石蜡切片染色。

染色结果：

含DNA的区域	紫红色

阴性对照：将同样切片经上述步骤，只有步骤 4 改为入预热的蒸馏水，孵育 15min。结果为染色阴性。

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

2. 注意Schiff 染色液的纯净程度，若变浅粉红加入适量的试剂A（2%以下）混匀，试剂变无色后使用，试剂完全变红则弃用。
3. 去除切片上多余Schiff染色液的方法以SO_2水洗为好。使用过程中应及时拧紧瓶盖，防止SO_2气体溢出。
4. 应做阴性对照试验。

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SO_2 Solution

Cat: G3920
Size: 2×100mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent	2×100mL	Storage
Reagent(A)：Sodium Disulfite Solution	100mL	RT, avoid light
Reagent(B)：Weak Acid Solution	100mL	RT
Before use, mix equal amounts of A and B to form SO_2 Working Solution. It is ready to use.		

Introduction

The principle of Feulgen Stain is that after DNA is hydrolyzed by mild weak acid (such as hydrochloric acid), the glycoside bond between purine base and deoxyribose is opened, and the phosphate 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 purplish red.

After dyeing, wash the tablets with SO_2 Solution to remove the excess non-specific pigment and diffuse dye.Make it more clear and clean.

Protocol(*for reference only*)

（一）Paraffin section staining

1. Tissue fixation: Carnoy fixative is better, 10% formalin can also be used, it is not suitable to use Bouin fixative.
2. Dewax to distilled water.
3. Soak the section in Weak Acid Working Solution (self prepared) at room temperature.
4. Soak the section in Weak Acid Working Solution preheated to 60 ℃ and incubate for 8min.
5. Place the section into Schiff Reagent and stain at room temperature for 30-60min.
6. In the above dyeing process, It is need to prepare SO_2 Working Solution.
7. Wash the section with fresh SO_2 Working Solution for 3 times, each time for 90s.
8. Wash in distilled water. After series of ethanol dehydration. Transparent by xylene and seal.

（二）Frozen section staining

1. Frozen section pretreatment:Fix with Carnoy Fixation for 10min.
2. Dehydration by absolute ethanol step by step down to distilled water.
3. The remaining steps are the same as the above paraffin staining.

Result

Nucleus DNA	Red Purple

Negative control

Take the same section and follow the above steps, only step 5 changes into "**at room temperature distilled water and incubate for 15min**".The result is negative for Nucleus DNA .

Note

1. Hydrolysis time is important and the hydrolysis time of different fixatives is different.

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

2. Pay attention to the purity of Schiff Regent. If it becomes light pink, add less than 2%V/V Reagent(A) to make it refresh. If it turns red, discard it.
3. The best way to remove the superfluous Schiff Reagent is washing in SO2 Solution.Working solution should be prepared before use. The cap shall be tightened in time to prevent SO2 gas from overflowing.
4. Negative control test should be done.