V02

黑色素染色试剂盒(硫酸亚铁法)

货号：G2031
规格：3×50mL/3×100mL
保存：室温，避光保存，有效期 1 个月。

产品组成：

名称	3×50mL	3×100mL	保存
试剂(A)：硫酸亚铁溶液	50mL	100mL	室温
试剂(B)：酸性铁氰化钾溶液	50mL	100mL	室温，避光
试剂(C)：核固红染色液	50mL	100mL	室温，避光

产品介绍：

黑色素属于非血源性内生色素，是一组由黑色素母细胞产生的颜色从浅棕色到黑色的色素。这种色素通常出现在皮肤表皮、眼睛、大脑的黑质和毛囊中。黑色素有一个显著的物理性质，由于黑素体中已形成的黑色素与蛋白质紧密结合，黑色素完全不溶解于大多数有机溶剂。黑色素另一个物理性质是能够被强氧化剂缓慢漂白。在病理情况下，这种色素也可出现在良性痣细胞瘤中和恶性黑色素瘤中。

许多方法可用于识别黑色素和黑色素生成细胞，如还原法：Masson-Fontana 银技术和 Schmorl 三价铁-铁氰化钾还原实验、酶法(如多巴反应)、荧光法、免疫组织化学等。硫酸亚铁法又称亚铁离子摄取法，本染色试剂盒适用于石蜡切片，采用中性福尔马林固定效果较好。

自备材料：

10%福尔马林固定液、蒸馏水

操作步骤：(*仅供参考*)

试剂(C)：核固红染色液可能会由于絮凝产生悬浮物或少量沉淀，建议取上清使用或沸水浴 5-10min 后晾至 30-40℃使用。（见注意事项 2）

1、 10%中性福尔马林固定，常规脱水，浸蜡，包埋，切片。
2、 将实验切片及对照切片脱蜡至蒸馏水。
3、 硫酸亚铁溶液室温浸染切片 1h。
4、 蒸馏水多次浸洗(5-6 次)，每次 1-2min。
5、 酸性铁氰化钾溶液室温浸染 30min。
6、 蒸馏水处理 3-4 次，每次 1min。
7、 滴加核固红染色液复染核 2-5min。
8、 蒸馏水冲洗 2 次。
9、 常规脱水、透明、中性树胶封固。

染色结果：

黑色素	深绿色
细胞核	红色

注意事项：

1、 该染色方法是黑色素特有的，具有很好的鉴别诊断作用。
2、 试剂(C)：核固红染色液为胶体性质溶液，低温（低于 25℃）保存或长期储存由于絮凝产生悬浮物或少量沉淀，属于正常现象，一般不影响使用。如移液器吸取观察到明显浑浊，可拧紧瓶盖沸水浴 5-10min 重新制备分散均匀的胶体溶液来恢复使用。
3、 应当避免使用铬酸盐和氧化汞等固定液。
4、 此方法不能使三价铁和脂褐素染色。
5、 核固红复染时，应根据具体切片摸索染色时间。
6、 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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Melanin Stain Kit(Ferroc Sulfate Method)

Cat: G2031
Size: 3×50mL/3×100mL
Storage:RT, avoid light, valid for 1 month.

Kit Components

Reagent	3×50mL	3×100mL	Storage
Reagent(A): Ferroc Sulfate Solution	50mL	100mL	RT
Reagent(B): Acid Potassium Ferricyanide Solution	50mL	100mL	RT, avoid light
Reagent(C): Nuclear Fast Red Solution	50mL	100mL	RT, avoid light

Introduction

Melanin is a kind of Nonhematogenous endogenous pigment, which is a group of pigments from light brown to black. This pigment is usually found in the skin, eyes, the substantia nigra of the brain and hair follicles. Melanin has a significant physical property. Because the melanin formed in melanosome is closely bound with protein, melanin is completely insoluble in most organic solvents. Another physical property of melanin is that it can be slowly bleached by strong oxidant. Under pathological condition, this kind of pigment can also appear in benign nevus cell tumor and malignant melanoma.

Many methods can be used to identify melanin and melanogenesis cells, such as reduction method: Masson Fontana silver method and Schmorl trivalent iron potassium ferricyanide reduction experiment, enzyme method (such as DOPA reaction), fluorescence method, immunohistochemistry, etc. Ferrous sulfate method, also known as ferrous ion uptake method, is suitable for paraffin section, and neutral formalin is better for fixation.

Self Provided Materials

10% Formalin Fixative, Distilled Water

Protocol(*for reference only*)

Reagent(C): Nuclear Fast Red Solution may produce suspended solids or a small amount of precipitation due to flocculation. It is recommended to take supernatant or boil water bath for 5-10min and then air it to 30-40 ℃. (see Note 2)

1. Fix with 10% neutral formalin, routinely dehydrate and embed, cut into slices. The thickness of the slice is recommended to be 3-5um.
2. Deparaffinize experimental and control sections to distilled water.
3. Incubate in Ferroc Sulfate Solution for 1h.
4. Wash with distilled water for five-six times fifth and each time for 1-2min.
5. Incubate with Acid Potassium Ferricyanide Solution for 30min.
6. Wash with distilled water for three-four times fifth and each time for 1min.
7. Re-dye the nucleus with Nuclear Fast Red Solution for 2-5min.
8. Wash with distilled water twice.
9. Conventional dehydration and transparency, seal with resinene.

Result

Melanin	Dark Green
Nucleus	Red

Note

1. This staining method is unique to melanin and has a good differential diagnosis effect.
2. Reagent(C): Nuclear Fast Red Solution is a colloidal solution, which is stored at low temperature (lower than 25 ℃) or stored for a long time. Suspended solids or a small amount of precipitation are generated due to flocculation, which is a normal phenomenon and generally does not affect the use. If the colloid solution is evenly dispersed in the boiling bath, tighten the bottle cap for 5-10min to recover the turbid solution.
3. Fixatives such as chromate and mercuric oxide should be avoided.
4. This method can not make trivalent iron and lipofuscin staining.
5. The staining time for Nuclear Fast Red Solution should be explored according to the specific sections.
6. For your safety and health, please wear experimental clothes and disposable gloves.