V02

普鲁士蓝染色试剂盒(三价铁，增强型)

货号：G1428
规格：4×10mL/4×20mL
保存：2-8℃，避光保存，有效期 1 年。

产品组成：

试剂名称		4×10mL	4×20mL	保存
试 剂 (A):Perls 染色工作液	试剂(A1):Perls A 液	5mL	10mL	室温，避光
	试剂(A2):Perls B 液	5mL	10mL	室温
临用前按照 1：1 混匀即为 Perls 染色工作液。				
试剂(B)：孵育液		10mL	20mL	2-8℃，避光
试剂(C)：浓缩增强液	试剂(C1):增强 A 液	0.5mL	1mL	2-8℃，避光
	试剂(C2):增强 B 液	0.5mL	1mL	2-8℃，避光
临用前按照 C1:C2:1×PBS=1:1:18 的比例混匀配制增强工作液，现配现用。				
试剂(D):复染液		10mL	20mL	室温，避光

产品介绍：

普鲁士蓝反应(Prussian Blue Reaction)是一种对含有颗粒铁的细胞或组织中的非血红素铁(含铁血黄素、铁转运蛋白、铁沉积等)进行染色的方法。在缺血性贫血、溶血性贫血、血色素沉着症和血色素代谢异常等疾病验证方面起重要作用，联合其他增强试剂也可用于铁死亡模型的检测。

普鲁士蓝反应受限于反应原理和成像设备精度仅能用于铁过载或铁沉积染色观察，对于含铁不丰富样本或非铁过载型铁死亡模型染色效果较差。普鲁士蓝染色试剂盒(三价铁，增强型)在常规普鲁士蓝染色的基础上引入了增强剂，利用级联放大的原理对阳性信号进行了放大显示，适用于含铁较不丰富或铁沉积较少的组织三价铁染色。

自备材料：

恒温箱或水浴锅、湿盒、1×PBS、蒸馏水

操作步骤：(*仅供参考*)

1. 组织石蜡包埋切成 3-7um 的切片，切片常规脱蜡复水。
2. 试剂盒从冰箱取出复温 20min 至室温(25-30℃)，湿盒注水置于 37℃恒温箱预热 20min。
3. 按照 1：1 的比例配置 Perls 染色工作液，滴加到切片上至完全覆盖组织，置于湿盒内 37℃孵育 20min。
4. 取出切片，蒸馏水轻轻冲洗 3 次，每次 10s。
5. 切片滴加孵育液至完全覆盖组织，置于湿盒内 37℃孵育 10-20min。(见注意事项 4)
6. 取出切片，1×PBS 轻轻浸洗 3 次，每次 60s。按照 C1:C2:1×PBS=1：1：18 的比例配置工作液，稀释后的工作液可在 2-8℃保存 1 周。
7. 切片滴加增强工作液至完全覆盖组织，置于湿盒内 37℃孵育 10-20min。(见注意事项 5)
8. (可选)取出切片，1×PBS 轻轻浸洗 3 次，每次 5s，滴加复染液染色 3-5min。
9. 蒸馏水浸洗 10min，梯度乙醇脱水，二甲苯透明，中性树胶封片。

染色结果：

含铁血黄素、三价铁	棕黄色到棕褐色
细胞核	浅蓝色
胞质	浅棕色或无色

注意事项：

1. 避免使用酸性固定液和螯合剂处理组织，铬酸盐处理也会影响铁的存在。
2. 所有工作液久置容易失效，建议现用现配，在 2 小时内使用完毕。

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

3. 孵育工作液孵育时间建议参考组织大小和细胞密度进行适当调整。
4. 增强工作液处理建议镜下控制着色程度，当阳性细胞着黄棕色到棕黑色时即可。
5. 如无法保证 37℃恒温处理可根据室温适当延长或缩短处理时间。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

相关产品：

G1420 普鲁士蓝染色试剂盒(中性红法)
G1422 普鲁士蓝染色试剂盒(核固红法)
G1424 普鲁士蓝染色试剂盒(伊红法)
G1426 普鲁士蓝染色试剂盒(细胞专用)
G1429 普鲁士蓝染色试剂盒(二价铁，增强型)
G3310 lillie 三价铁染色试剂盒
G3320 lillie 二价铁染色试剂盒

V02

Prussian Blue Iron Stain Kit (Ferric Iron, Enhance With DAB)

Cat: G1428
Size: 4×10mL/4×20mL
Storage: 2-8℃, avoid light, valid for 1 year.

Kit components

Reagent		4×10mL	4×20mL	Storage
Reagent(A):Perls Working Solution	A1:Perls Solution A	5mL	10mL	RT, avoid light
	A2:Perls Solution B	5mL	10mL	RT
Before use, mix A1 with A2 in equal amount to prepare Perls Working Solution.				
Reagent(B):Incubation Solution		10mL	20mL	2-8℃, avoid light
Reagent(C): Enhanced Solution	C1:Enhanced Solution A	0.5mL	1mL	2-8℃, avoid light
	C2:Enhanced Solution B	0.5mL	1mL	2-8℃, avoid light
Before use, mix C1, C2 with1×PBS in 1:1:18 ratio to prepare Enhanced Working Solution, it is ready to use.				
Reagent(D):Redyeing Solution		10mL	20mL	RT, avoid light

Introduction

Perl's iron staining method is one of the common histochemical methods to detect non heme iron in cells and tissues. It detects iron ions in bone marrow, liver, spleen, kidney and other tissues and cells by forming blue Prussian blue precipitation. However, for organs that are not rich in iron, such as brain tissue, this method is often ineffective because there is too little blue precipitation. Prussian Blue Iron Stain Kit (Enhance With DAB) uses the principle of cascade amplification to amplify and display the positive signal. It is suitable for iron staining in tissues with less iron or less iron deposition.

Self Provided Materials

Thermostat or water bath, wet box, 1 × PBS, distilled water

Protocols*(for reference only)*

1. Cut the tissue in paraffin sections of 3-7μm. Dewax the slices to water routinely.
2. Take out the kit from the refrigerator and rewarm it for 20min to room temperature (25-30 ℃), inject water into the wet box and preheat it in a 37 ℃ incubator for 20min.
3. Prepare Perls Working Solution in the ratio of 1:1, drop it onto the slices until the tissues are completely covered, and incubate in a wet box at 37 ℃ for 20min.
4. Take out the slices, gently rinse them with distilled water for three times, 10s each time.
5. Drip Incubation Solution onto the slices until the tissue are completely covered, and incubate in a wet box at 37 ℃ for 10-20min. (*see note 4*)
6. Take out the slices, gently soak with 1×PBS for three times, 60s each time. According to C1: C2:1×PBS=1:1:18 to prepare Enhanced Working Solution, the working solution can be stored at 2-8℃ for one week.
7. Drip Enhanced Working Solution onto the slices until the tissue are completely covered, and incubate in a wet box at 37 ℃ for 10-20min. (*see note 5*)
8. Take out the slice, gently soak with 1×PBS for three times, 5s each time, and stain with Redyeing Solution for 3-5min.
9. Soak in distilled water for 10min.
10. Dehydrate in gradient ethanol, transparent by xylene and seal with resinene.

Result

Hemosiderin or Ferric Ions	Yellow Brown or Brown
Nucleus	Light Blue
Cytoplasm	Light Brown or Colorless

Note

1. After getting the kit, it is recommended to take out the Reagent C1 and repack it according to the single use

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amount or directly place it in the refrigerator at - 20 ℃ for storage, and place the remaining reagent of the kit in the refrigerator at 2-8 ℃.

2. Avoid using acidic fixatives to treat tissues. Chromate treatment will also affect the presence of iron.
3. All working solution are easy to lose effect after a long time. It is recommended to use and prepare them now and use them within 2 hours.
4. The incubation time of Incubation Working Solution should be adjusted appropriately due to tissue size and cell density.
5. To enhance the treatment of working solution, it is recommended to control the staining degree under the microscope, when the positive cells are yellow brown to brown black.
6. If the constant temperature treatment at 37 ℃ cannot be guaranteed, the treatment time can be appropriately extended or shortened according to the room temperature.
7. For your safety and health, please wear experimental clothes and disposable gloves.

Related products

G1420 Prussian Blue Iron Stain Kit (With Neutral Red)
G1422 Prussian Blue Iron Stain Kit (With Nuclear Fast Red)
G1424 Prussian Blue Iron Stain Kit (With Eosin)
G1426 Prussian Blue Iron Stain Kit (For Cells)
G3310 Lillie's Ferric Iron Stain Kit
G3320 Lillie's Ferrous Iron Stain Kit