普鲁士蓝染色试剂盒（增强型）

货号： G1428
规格： 4×2mL（试用装）/4×10mL/4×20mL
保存： -20℃, 避光保存, 有效期 6 个月。

产品组成：

试剂名称		4×2mL	4×10mL	4×20mL	保存
试剂 (A):Perls 染色工作液	试剂(A1):Perls A 液	1mL	5mL	10mL	室温，避光
	试剂(A2):Perls B 液	1mL	5mL	10mL	室温
临用前按照 1：1 混匀即为 Perls 染色工作液。					
试剂(B)：孵育工作液	试剂(B1):孵育浓缩液	0.2mL	1mL	2×1mL	2-8℃，避光
	试剂(B2):孵育稀释液	1.8mL	9mL	18mL	室温
临用前按照 1：9 混匀即为孵育工作液。					
试剂(C)：增强工作液	试剂(C1):增强 A 液	1.9mL	9.5mL	19mL	-20℃，避光
	试剂(C2):增强 B 液	0.1mL	0.5mL	1mL	2-8℃，避光
临用前按照 19：1 的比例混匀即为增强工作液。					
试剂(D):复染液		2mL	10mL	20mL	室温，避光

产品说明：

Perl's 铁染色法是检测细胞和组织中非血红素铁的常用组织化学方法之一，通过形成蓝色的普鲁士蓝沉淀检测骨髓及肝、脾、肾等组织细胞中的铁离子，然而，对于含铁不丰富的器官，如脑组织，运用此法常常由于蓝色沉淀形成过少而无效。普鲁士蓝染色试剂盒（增强型）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，蒸馏水轻轻冲洗三次，每次 10s，按照 1：9 配置孵育工作液。
5. 切片滴加孵育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 4）
6. 取出切片，1×PBS 轻轻浸洗三次，每次 60s，按照 19：1 的比例配置增强工作液。
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. 取出切片，1×PBS 轻轻浸洗三次，每次 5s，滴加复染液染色 3-5min。
9. 蒸馏水浸洗 10min。
10. 梯度乙醇脱水，二甲苯透明，中性树胶封片。

染色结果：

常规细胞核	红紫色到蓝色
阳性细胞	黄棕色到黄褐色

胞质	浅紫色或无色

注意事项：

1. 收到试剂盒之后建议将试剂(C1)取出，按照单次使用量分装或直接放置-20℃冰箱保存，剩余试剂盒放置 2-8℃冰箱保存。
2. 避免使用酸性固定液处理组织，铬酸盐处理也会影响铁的存在。
3. 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。
4. 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。
5. 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。
6. 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。
7. 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)
G3310　lillie 三价铁染色试剂盒
G3320　lillie 二价铁染色试剂盒

Prussian Blue Iron Stain Kit (Enhance With DAB)

Cat: G1428
Size: 4×2mL(free sample)/4×10mL/4×20mL
Storage: -20°C, avoid light, valid for 6 months.

Kit components

Reagent		4×2mL	4×10mL	4×20mL	Storage
Reagent(A):Perls Working Solution	A1:Perls Solution A	1mL	5mL	10mL	RT, avoid light
	A2:Perls Solution B	1mL	5mL	10mL	RT
Before use, mix A1 with A2 in equal amount to prepare Perls Working Solution.					
Reagent(B):Incubation Working Solution	B1:Incubation Stock Solution	0.2mL	1mL	2×1mL	2-8°C, avoid light
	B2:Incubation Diluent	1.8mL	9mL	18mL	RT
Before use, mix B1 with B2 in equal amount to prepare Incubation Working Solution.					
Reagent(C):Enhanced Working Solution	C1:Enhanced Solution A	1.9mL	9.5mL	19mL	-20°C, avoid light
	C2:Enhanced Solution B	0.1mL	0.5mL	1mL	2-8°C, avoid light
Before use, mix C1 with C2 as the ratio of 19:1 to prepare Enhanced Working Solution.					
Reagent(D):Redyeing Solution		2mL	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 °C), inject water into the wet box and preheat it in a 37 °C 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 °C for 20min.
4. Take out the slices, gently rinse them with distilled water for three times, 10s each time, and prepare the Incubation Working Solution according to the ratio of 1:9.
5. Drip Incubation Working Solution onto the slices until the tissuea are completely covered, and incubate in a wet box at 37 °C for 10-20min. (*see note 4*)
6. Take out the slices, gently soake with 1×PBS for three times, 60s each time, and prepare Enhanced Working Solution according to the ratio of 19:1.
7. Drip Enhanced Working Solution onto the slices until the tissuea are completely covered, and incubate in a

wet box at 37 ℃ for 10-20min. (*see note 5*)

8. Take out the slice, gently soake 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

Regular nucleus	Red Purple to Blue
Positive cell	Yellowish Brown to dark Yellowish Brown
Cytoplasm	Light Purple 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 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*