V02

Lillie 二价铁染色试剂盒

货号：G3320
规格：2×50mL
保存：室温，避光保存，有效期 1 年。

产品组成：

名称		2×50mL	保存
试剂(A):Lillie 染色工作液	A1:Lillie 二价铁 A 液	25mL	室温
	A2:Lillie 二价铁 B 液	25mL	室温，避光
临用前，取 A1、A2 等量混合即为 Lillie 染色工作液，不宜提前配制。			
试剂(B): 核固红染色液		50mL	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。

普鲁士蓝反应最早被 Perls 提出，因此最常被称为 Perl 法铁染色，但是自 1867 年提出以来有众多化学家和病理学家对该反应进行优化，其中优化比较完善使用也比较多的一种称为 Lillie 法，能够对二价铁和三价铁进行分别标记。本试剂盒即为即用型的 Lillie 法二价铁染色试剂盒，采用优质原料配制，试剂稳定，染色特异性良好。

操作步骤：*(仅供参考)*

1. 组织推荐固定于中性福尔马林固定液(10%)或其他非酸性固定液中，常规脱水包埋。（*见注意事项 1*）
2. 石蜡切片厚度 5μm，常规脱蜡至水。冰冻切片推荐厚度 10μm，常规复温复水。（*见注意事项 2*）
3. 切片滴加 Lillie 染色工作液染色 25～30min，蒸馏水冲洗 2～5min。
4. 滴加核固红染色液覆盖切片，淡染细胞核 5～10min，蒸馏水洗 1～5s。（*见注意事项 3*）
5. 常规脱水透明，中性树胶封固。

染色结果：

二价铁阳性位点	蓝色或蓝绿色
细胞核、其他组织	红色

对照(*可选*)：

取相同对照切片脱蜡至水，1%盐酸水溶液室温处理 20min 后正常染色。结果为染色阴性。

取少量亚铁盐配成不同浓度的水溶液试样（常用 0.1%硫酸亚铁水溶液），滴入一滴染色工作液应观察到明显蓝色沉淀出现，结果为试剂盒阳性。

取少量铁盐配成不同浓度的水溶液试样（常用 0.1%氯化铁或硫酸铁水溶液），滴入一滴染色工作液应观察到无明显变化或有微量变色，结果为试剂盒阴性。

注意事项：

1. 该染色法组织取材应避免使用酸性固定液或螯合剂处理导致铁离子丢失，推荐使用 G2161-中性福尔马林固定液(10%)或 P1110-4%组织细胞固定液进行组织固定。
2. 该染色法适用于石蜡切片、冰冻切片和树脂切片。但由于二价铁不稳定，制片过程中须避免接触氧化剂或长时间处理，因此通常建议组织灌注染色后取材制备切片，如无法灌注建议制备速冻切片染色。
3. 试剂(B): 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。
4. 整个操作过程中须避免铁离子污染，清洗用水以蒸馏水为宜，因自来水常内含铁质。
5. 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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Lillie's Ferrous Iron Stain Kit

Cat: G3320
Size: 2×50mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent		2×50mL	Storage
Reagent(A):Lillie Stain Solution	A1:Lillie's Ferrous Iron Solution A	25mL	RT
	A2:Lillie's Ferrous Iron Solution B	25mL	RT, avoid light
Mix A1,A2 in equal to form Lillie Stain Solution before use, which can not keep for long.			
Reagent(B): Nuclear Fast Red Solution		50mL	RT, avoid light

Introduction

The Prussian Blue Reaction is a method of staining non heme iron (containing heme, iron transporters, iron deposition, etc.) in cells or tissues containing granular iron. It plays an important role in the validation of diseases such as ischemic anemia, hemolytic anemia, hemochromatosis, and abnormal hemoglobin metabolism, and can also be used in conjunction with other enhancers for the detection of iron death models.

The Prussian blue reaction was first proposed by Perls and is therefore most commonly referred to as the Perl iron staining method. However, since its proposal in 1867, numerous chemists and pathologists have optimized this reaction, among which the Lillie method, which is more widely used and well optimized, can label divalent and trivalent iron separately. This kit is a ready to use Lillie method ferrous iron staining kit, prepared with high-quality raw materials, with stable reagents and good staining specificity.

Protocol(*for reference only*)

1. The organization recommends fixation in neutral formalin fixative (10%) or other non acidic fixative, conventional dehydration and embedding. (*See Note 1*)
2. Cut the paraffin section in 5μm thickness. Conventional dewaxing to water. The recommended thickness for frozen sections is 10μm. Regular rewarming and rehydration. (*See Note 2*)
3. Stain with Lillie Stain Solution for 25-30min and rinsed with distilled water for 2-5min.
4. Re-dye with Nuclear Fast Red for 5-10min. Rinse with distilled water for 1-5s.(*See Note 3*)
5. Conventionally dehydrate and transparent, then seal with resinene.

Result

Ferric Iron	Blue or Bluish Green
Background	Red

Negative Control(*Optional*)

Take the same control section and dewax it to water, then treat it with a 1% hydrochloric acid solution at room temperature for 20min before staining normally. The result is negative for staining.

Take some ferrous salt and prepare an aqueous solution samples (commonly 0.1% ferrous sulfate aqueous solution), and drop a drop of staining solution, the result is positive for kit.

Take some iron salt and prepare an aqueous solution samples (commonly 0.1% ferric chloride or ferric sulfate aqueous solution). Drop a drop of the staining solution, the result is a negative for kit.

Note

1. This staining method for tissue sampling should avoid using acidic fixative or chelating agent treatment that may cause iron ion loss. It is recommended to use G2161-Neutral Buffered Formalin Fixative, 10% or P1110-Paraformaldehyde,4% for tissue fixation.
2. This staining method is applicable to paraffin sections, frozen sections, and resin sections. However, due to the instability of divalent iron, it is necessary to avoid contact with oxidants or prolonged treatment during the production process. Therefore, it is usually recommended to prepare sections after tissue perfusion staining. If perfusion is not possible, it is recommended to prepare frozen sections for staining.
3. Reagent (B): Nuclear Fast Red Solution is a colloidal solution.Long term storage at low temperatures (below 25 ℃) will produce a small amount of precipitation, which is a normal phenomenon and generally does not affect its use. If obvious turbidity is observed, it can be restored to use by boiling water for 5-10 min.
4. During the entire operation process, it is necessary to avoid iron ion pollution, and distilled water is preferred for cleaning, as tap water often contains iron.
5. For your safety and health, please wear laboratory clothes and disposable gloves.