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): 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：

含铁血黄素(Hemosiderin)是一种血红蛋白源性色素，为金黄色或棕黄色颗粒，因其含铁，且为金黄色，故称为含铁血黄素。当红细胞被巨噬细胞吞噬后，在溶酶体酶的作用下，血红蛋白被分解为不含铁的橙色血质和含铁的含铁血黄素。普鲁士蓝反应(Prussian Blue Reaction)又称为含铁血黄素染色，即铁血黄素经过亚铁氰化钾和稀酸处理后可以产生蓝色，常见于吞噬细胞或间质内，主要显示三价铁盐。在极少数情况下，铁存在于其还原态的亚铁之中，Lillie 法是显示二价铁的很好的方法，其特异性较好。

操作步骤：*(仅供参考)*

试剂(B): 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. 组织固定于 10%中性福尔马林或其他碱性固定液，常规脱水包埋。
2. 切片厚度 4μm，常规脱蜡至水。
3. 切片入 Lillie 染色液(见注意事项 4)，浸染 25～30min，蒸馏水冲洗 2～5min。
4. 入核固红染色液，淡染细胞核 5～10min，馏水冲洗 1～5s。
5. 常规脱水透明，中性树胶封固。

染色结果：

二价铁	深藤氏蓝
细胞核、其他组织	红色

阴性对照(*可选*):

取相同对照切片脱蜡至水，直接染色核固红。结果为阴性。

注意事项：

1. 该染色法适用于石蜡切片、冰冻切片和树脂切片。系列乙醇应经常更换新液。
2. 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。
3. 组织固定建议采用 10%中性福尔马林，酸性固定液会造成铁离子的流失。
4. 整个操作过程中容器要避免铁离子污染，洗切片和容器时以蒸馏水为宜，因自来水内含铁质。
5. Lillie 染色液染色时，应根据样本情况调整着色时间。冰冻切片和细胞的染色，最好根据具体情况摸索实验条件。
6. 所有切片都应使用同一个阳性对照切片，选择适合的对照非常重要。肺组织是一个很好的对照样本，包含相当数量的铁阳性巨噬细胞(心衰细胞)。
7. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

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

Hemosiderin is a hemoglobin derived pigment, which is golden yellow or brownish yellow particles. Because it contains iron and is golden yellow, it is called hemosiderin. When the red blood cells are engulfed by macrophages, under the action of lysosomal enzymes, hemoglobin is broken down into iron free orange blood and iron-containing hemosiderin. Perls Prussian blue reaction is also called hemosiderin staining, that is, hemosiderin can produce blue after being treated with potassium ferrocyanide and dilute acid, which is common in phagocytes or interstitium, mainly showing trivalent iron salt. In very few conditions, iron exists in the reduced state of ferrous, Lillie method is a good method to show the divalent iron, and its specificity is good.

Protocol(*for reference only*)

Reagent(B): 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 the tissue in 10% NBF or other alkamine fixative,then conventionally dehydrate and embed.
2. Cut the tissue into 4μm and conventionally dewax and hydrate.Rinse with distilled water for 1 min.
3. Dye with Lillie Stain Solution for 25-30min.(See Note 4).Rinse with distilled water for 2-5min.
4. Re-dye with Nuclear Fast Red for 5-10min.Rinse with distilled water for 1-5s.
5. Conventionally dehydrate and transparent,then seal with resinene.

Result

Ferric Iron	Deep Prussian blue
Background	Red

Negative Control(*Optional*)

Take a serial section dewax and hydrate,then dye with Nuclear Fast Red Solution to get the negative result.

Note

1. This method is suitable for paraffin section, frozen section and resin section.In order to get better result,dewaxing the section thoroughly is needed.Series ethanol should be replaced frequently.
2. Reagent(B): 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. 10% NBF is recommended to use in this method.The acid fixative will make bad effect to the protecting of iron.
4. Keep all container clean and avoid occuring exceed iron by operation.
5. All sections should use the same positive control section, so it is very important to select the appropriate control. Autopsy lung tissue is a good control, containing a considerable number of iron positive macrophages (heart failure cells).
6. For frozen sections and cell staining, it is best to explore the experimental conditions according to the specific situation.
7. For your safety and health, please wear experimental clothes and disposable gloves.