普鲁士蓝染色液（细胞专用）

货号： G1426
规格： 2×20mL/2×50mL
保存： 室温，避光保存，有效期 1 年。

产品组成：

名称		2×20mL	2×50mL	保存
试剂(A): Perls染色工作液	A1: Perls染色液A	10mL	25mL	室温，避光
	A2: Perls染色液B	10mL	25mL	室温
临用前，取A1、A2等量混合即为Perls stain，不宜提前配制。				
试剂(B): Perls复染液		20mL	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，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。Perls 普鲁士蓝反应(Prussian blue reaction)又称为含铁血黄素染色，即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，其染色原理在于亚铁氰化钾溶液使三价铁离子从蛋白质中被稀盐酸分离出来，三价铁与亚铁氰化钾反应，生成一种不溶解的蓝色化合物即三价铁的亚铁氰化物。

普鲁士蓝染色液主要用于显示细胞中铁离子，常见于吞噬细胞内，可以很好地区分含铁血黄素与其他色素。该染色液稳定性好、可以长期保存、不易产生沉淀、应用范围广，可以进行复染。

自备材料：

固定液：甲醇或4%多聚甲醛、蒸馏水

操作步骤：*(仅供参考)*

1、 制备血液、骨髓涂片，甲醇固定5min或4%多聚甲醛固定10～20min。
2、 用配制好的Perls染色工作液(见注意事项4)滴满涂片，37℃孵育30min。 自来水冲洗2次，每次2min。
3、 用Perls复染液滴染于涂片，复染30s～1min。
4、 水洗、晾干、镜检。

染色结果：

铁颗粒	蓝色
幼红细胞核	红色

细胞外铁分级：

-	无蓝色颗粒
+	有少量铁粒或偶见铁小珠
2+	有较多铁粒或铁小珠
3+	有很多铁粒、铁小珠和少数小块状
4+	有极多铁粒、铁小珠，并有许多小块

细胞内铁：计数100个有核红细胞，记录细胞质中含有蓝色铁粒细胞(铁粒幼红细胞)的百分率。环形铁粒幼红细胞是指幼红细胞含铁粒＞6绕核径2/3以上者。

注意事项：

1、 避免使用酸性固定剂，铬酸盐处理也会妨碍铁的保存。
2、 整个操作过程中容器要干净，避免用金属铁制品，洗切片和容器时以蒸馏水为宜，因普通水内含铁质。
3、 细胞外铁需用含有骨髓小粒的涂片。
4、 Perls stain染色时，应根据样本情况调整着色时间。
5、 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Prussian Blue Iron Stain Kit (For Cells)

Cat: G1426
Size: 2×20mL /2×50mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent		2×20mL	2×50mL	Storage
Reagent(A): Perls Stain	A1：Perls Stain A	10mL	25mL	RT, avoid light
	A2：Perls Stain B	10mL	25mL	RT
Before use, mix equal parts of A1 and A2 to form Perls Stain. It is not suitable to prepare in advance.				
Reagent(B): Perls Counterstain Solution		50mL	100mL	RT, avoid light

Introduction

Hemosiderin is a hemoglobin derived pigment. Because it contains iron and 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, also known as hemosiderin staining, can produce blue after being treated with potassium ferrocyanide and dilute acid, which is common in the interstitium of phagocytes, mainly showing ferric iron salts. Its dyeing principle is: potassium ferrocyanide solution separates the ferric iron from the protein by dilute hydrochloric acid, and the ferric iron reacts with potassium ferrocyanide to form an insoluble blue compound named Prussian blue.

Perls Stain is often used to display various hemorrhagic lesions in local tissues, and it is common in phagocytes. Perls reaction can be used to determine the deposition of hemosiderin, and this staining method can distinguish hemosiderin from other pigments. The dyeing solution has good stability, can be preserved for a long time, is not easy to produce precipitation, has a wide range of applications, and can be used for re-dyeing.

Self Provided Materials

Fixative: methanol or 4% paraformaldehyde, Distilled water

Protocol(*for reference only*)

1. Make blood or bone marrow smear, then fix in methanol for 5min or fix in 4% PFA for 10-20 min.
2. Drop prepared Perls Stain and fully cover the smear(***see note 4***), then incubate at 37℃ for 30min.Rinse in tap water twice for each time 2min.
3. Counterstain with Perls Counterstain Solution for 30s-1min.
4. Wash, air dry, view under the microscope.

Result

Iron particles	Blue
Immature erythrocyte nucleus	Red

The lever of iron outside the cell:

-	No blue particles
+	A few iron particles or occasionally iron beads
2+	Some iron particles or iron beads
3+	Many iron particles , iron beads and few small blocks
4+	Most iron particles , iron beads and many small blocks

The iron inside the cell: count 100 nucleated red blood cells and the record the percentage of cells containing blue iron(iron erythroblasts) in the cytoplasm. The ring shaped erythroblasts refer to the erythroblasts that the lever is over 6 and surround more than 2 / 3 of the diameter around the nucleus.

Note

1. Avoid the use of acid fixatives, chromate treatment will also hinder the preservation of iron.
2. During the whole operation process, the container should be clean and avoid the use of metal iron products. When washing sections and containers, distilled water is suitable, because ordinary water contains iron.
3. The iron outside the cell needs smears containing bone smarrow particles.
4. When dyeing with Perls Stain, the time should be adjusted according to the sample situation.
5. For your health and safety, please wear the experimental clothes and disposable gloves.