V02

碱性蛋白染色试剂盒

货号：G2561
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)：酸性分化液		50mL	室温，避光
试剂(B)：碱性固绿染色液	试剂(B1)：碱性固绿 A	25mL	室温，避光
	试剂(B2)：碱性固绿 B	25mL	室温
临用前，取 B1、B2 等量混合，即为碱性固绿染色液，不宜提前配制。			

产品介绍：

固绿(Fast Green FCF)是一种特殊染料，只与带正电的蛋白质结合，从而使蛋白质染上颜色；而不与带负电的蛋白质结合。细胞内绝大多数蛋白质的等电点大于 2.6，当用低 pH 的固绿染色时，此时大部分酸性蛋白质都能与固绿结合染上颜色，从而显示细胞酸性蛋白。当用高 pH 固绿染色时，等电点大的那些碱性蛋白质带正电，与固绿结合染上颜色，从而显示细胞碱性蛋白。

本试剂盒使用碱性固绿法对组织或细胞蛋白进行染色，通过分析染色结果，推断总体蛋白和碱性蛋白在细胞中分布状况，如分布部位、分布量和存在状态等等。

自备材料：

玻片、中性福尔马林固定液、70%乙醇、水浴锅、恒温烘箱、显微镜

操作步骤：(*仅供参考*)

（一）对于贴壁细胞或爬片染色

1. 使用中性福尔马林固定液或组织细胞固定液室温固定 10-20min。
2. 按照培养基添加量的一半加入酸性分化液，烘箱 60℃孵育 30min，蒸馏水洗两次，每次 1min。
3. 吸去多余水分，滴加同分化液等量的碱性固绿染色液染色 5～15min，蒸馏水洗 30s，带水观察。

（二）对于血涂片或悬浮细胞染色

1. 取新鲜血液 1 滴滴于载玻片一端，推片，室温晾干。
2. 涂片浸入 70%乙醇中固定 5min，室温晾干。
3. 涂片浸入酸性分化液中，60℃水浴 30min。流水充分水洗，滤纸吸去残留水分。
4. 涂片浸入碱性固绿染色液染色 15-30min。流水冲洗，室温晾干。
5. 直接镜检或滴加中性树胶，加盖盖玻片进行封片观察。

染色结果：

细胞核大部分区域	绿色
细胞质、核仁	不着色

注意事项：

1. 血液涂片或骨髓涂片应厚薄均匀，以免影响染色效果。
2. 血细胞涂片染色要求新鲜全血或 EDTA 抗凝血。
3. 酸性分化液孵育后，冲洗应彻底，否则会干扰固绿的染色。
4. 染色过深可用甲醇或酒精适当脱色，最好不复染。
5. pH 值对染色有一定影响，载玻片应清洁、无酸碱污染，以免影响染色效果。
6. 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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Alkaline Protein Stain Kit, For Cell

Cat: G2561
Size: 2×50mL
Storage: RT, avoid light, valid for 1 year.

Kit Components

Reagent		2×50mL	Storage
Reagent(A): Acid Differentiation Solution		50mL	RT, avoid light
Reagent(B):Alkaline Fast Green Solution	B1: Alkaline Fast Green Solution A	25mL	RT, avoid light
	B2: Alkaline Fast Green Solution B	25mL	RT
Before use, mix B1 with B2 in equal amount to form Alkaline Fast Green Solution. It is ready to use.			

Introduction

Fast Green FCF is a special dye that only binds to positively charged proteins, thereby coloring them. When using low pH fixed green staining, most acidic proteins can bind with fixed green to stain, thereby displaying cellular acidic proteins. When using high pH fixed green staining, the alkaline proteins with higher isoelectric points carry a positive charge and bind with fixed green to stain, thus displaying the alkaline proteins of the cells.

This reagent kit uses the basic method to stain tissue or cellular proteins. By analyzing the staining results, the overall distribution of proteins and alkaline proteins in cells can be inferred, such as distribution location, quantity, and presence status.

Self Provided Materials

Slide, 70% ethanol, Water bath, Microscope.

Protocol(*for reference only*)

For Adherent Cells or Climbing Section Staining

1. The cells were fixed in neutral formalin or tissue cell solution at room temperature for 10-20 min.
2. Add Acid Differentiation Solution according to half of the added amount of medium, incubate in warm at 60 ℃ for 30min, and wash twice with distilled water for 1min each time.3. Absorb excess water.
3. Add the same amount of Alkaline Fast Green Solution to dye for 5-15 min, and wash with distilled water for 30 seconds.Observe with water.

For Blood Smear or Suspension Cell Staining

1. Take 1 drop of fresh blood to one end of the slide, push the slide and dry at room temperature.
2. Immerse the smear in 70% ethanol for 5min and dry it at room temperature.
3. Immerse the smear in Acid Differentiation Solution and incubate in 60 ℃ water bath for 30min.
4. Rinse with running water completely and absorb residual water by filter paper .
5. Immerse the smear in Alkaline Fast Green Solution for 15-30 min.
6. Rinse with running water and dry at room temperature.
7. View under the microscope directly or after sealing with resinene.

Result

Cytoplasm and Nucleolus	Green
Most areas of nucleus	Colorless

Note

1. The blood smear or bone marrow smear should be uniform in thickness to avoid affecting the staining effect.
2. Blood cell smear staining requires fresh whole blood or EDTA anticoagulant.
3. Wash thoroughly Acid Differentiation Solution, , otherwise it will interfere with the staining of fast green.
4. If the dye color is too deep, it can be decolorized properly with alcohol, and it is better not to be re-dyed.
5. The pH value has certain influence on the dyeing. The slide should be clean and free of acid and alkali pollution to avoid affecting the dyeing effect.
6. For your safety and health, please wear experimental clothes and disposable gloves.