V02

组织革兰氏染色试剂盒

货号：G3225
规格：5×50mL
保存：室温，避光，有效期 6 个月。

产品组成：

名称	5×50mL	保存
试剂(A): 结晶紫染色液	50mL	室温，避光
试剂(B): 卢戈碘液	50mL	室温，避光
试剂(C): 复红染色液	50mL	室温，避光
试剂(D): 分化液	50mL	室温
试剂(E): 复染液	50mL	室温，避光

产品介绍：

革兰氏染色法是丹麦医生 Christain Gram 于 1884 年所发明，是细菌学中广泛使用的一种鉴别染色法。通过此法染色可将细菌鉴别为革兰阳性菌(G+)和革兰阴性菌(G-)两大类。细菌的不同显色反应是由于细胞壁对乙醇的通透性和抗脱色能力的差异，主要是肽聚糖层厚度和结构决定的。

本染色试剂盒是从原有的革兰氏染色方法改良而来，特别适用于鉴别组织切片中的细菌感染。细菌被染成深紫色或者粉红色，在复染液的作用下组织背景着色为黄色，从而清晰的观察组织中存在的细菌。

操作步骤：*（仅供参考）*

1. 多聚甲醛常规固定，包埋后切 3-7μm 切片，脱蜡至水后进行以下染色步骤。
2. 结晶紫染色液染色 2min，自来水冲洗，去除多余染色液。
3. 卢戈碘液媒染 5min，自来水冲洗，去除多余碘，沥去多余水分但不完全晾干。
4. 使用 95%乙醇分化至无多余紫色洗脱，自来水冲洗终止分化。
5. 复红染色液染色 5min，自来水短暂清洗去除多余染色液。
6. 滴加分化液孵育 5min，自来水清洗，沥去多余水分但不完全晾干。
7. 95%乙醇快速清洗三次脱水。
8. 复染液快速染色 10-20s，无水乙醇清洗 5-10s。
9. 无水乙醇-二甲苯（无水乙醇和二甲苯 1:1 混合）脱水 5-10s。
10. 二甲苯透明，中性树胶封片。

染色结果：

革兰阳性细菌和细胞核	紫色
革兰阴性细菌	红色
背景	黄色

注意事项：

1. 菌体较小，需用 100 倍油镜观察结果。
2. 95%乙醇分化步骤如果滴加分化，应当多次更换新的 95%乙醇，直至无紫色脱出。
3. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

Tissue Gram Staining kit

Cat: G3225
Size: 5×50mL
Storage: RT, avoid light, valid for 6 months.

Kit Components

Reagent	5×50mL	Storage
Reagent A: Crystal Violet Staining Solution	50mL	RT, avoid light
Reagent B: Lugo iodine solution	50mL	RT, avoid light
Reagent C: Compound red staining solution	50mL	RT, avoid light
Reagent D: Differentiation fluid	50mL	RT
Reagent E: Counterstain	50mL	RT, avoid light

Introduction

The Gram stain, invented in 1884 by the Danish physician Christain Gram, is a widely used identification stain in bacteriology. By staining with this method, bacteria can be identified into two major groups, Gram-positive (G+) and Gram-negative (G-). The different coloring reactions of bacteria are due to differences in cell wall permeability to ethanol and resistance to decolorization, mainly determined by the thickness and structure of the peptidoglycan layer.

This staining kit is modified from the original Gram staining method and is particularly suitable for identifying bacterial infections in tissue sections. Bacteria are stained dark purple or pink, and the background of the tissue is colored yellow in the presence of a re-staining solution, allowing a clear view of the bacteria present in the tissue.

Protocols(*for reference only*)

1. Use paraformaldehyde to fix the sections normally, the embedded tissues were cut into 3-7μm thin slices, deparaffinized to distilled water, and then the following staining steps were performed.Crystalline violet staining solution was used to stain the sections for 2 min, rinsed with tap water, and excess staining solution was removed.
2. Crystalline violet staining solution was stained for 2min, rinsed with tap water to remove excess staining solution.
3. Mordant staining with Lugol's iodine solution for 5min, rinse with tap water, remove excess iodine, drain off excess water but not completely dry.
4. Use 95% ethanol to differentiate until no excess purple elution, rinse with tap water to terminate differentiation.
5. Stain with red staining solution for 5 min, wash briefly with tap water to remove excess staining solution.
6. Add the differentiation solution dropwise for 5min, rinse with tap water, drain off excess water but not completely dry.
7. 95% ethanol rapid wash three times dehydration.
8. Rapid staining with re-staining solution for 10-20s, washing with anhydrous ethanol for 5-10s.
9. Anhydrous ethanol-xylene (anhydrous ethanol and xylene 1:1 mixture) dehydration 5-10s.
10. Xylene transparent, neutral gum sealing.

Result

Gram-positive bacteria and nuclei	Purple
Gram-negative bacteria	Red
Background	Yellow

Note

1. The bacteria are small and the results need to be observed with a 100x oil microscope.
2. 95% ethanol differentiation step should be replaced with new 95% ethanol several times until no purple color comes off if the drop is differentiated.
3. For your safety and health, please wear lab coat and disposable gloves to operate.