V02

革兰氏染色试剂盒

货号：G1060
规格：4×10mL/4×100mL/4×500mL
保存：2-8℃，避光保存，有效期 1 年。

产品组成：

名称	4×10mL	4×100mL	4×500mL	保存
试剂（A）：结晶紫染色液	10mL	100mL	500mL	2-8℃
试剂（B）：番红染色液	10mL	100mL	500mL	2-8℃
试剂（C）：碘液	10mL	100mL	500mL	2-8℃，避光
试剂（D）：脱色液	10mL	100mL	500mL	室温

产品介绍：

革兰氏染色法是细菌学中广泛使用的一种鉴别染色法，通过结晶紫初染和碘液媒染后，在细胞壁内形成了不溶于水的结晶紫与碘的复合物，革兰氏阳性菌细胞壁较厚，肽聚糖网层次较多且交联致密，脱色液脱色时，肽聚糖脱水使孔径缩小，故保留结晶紫-碘复合物在细胞膜上，呈紫色。革兰氏阴性菌细胞壁薄、外膜层类脂含量高、肽聚糖层薄且交联度差，脱色后类脂外膜迅速溶解，缝隙加大，结晶紫与碘复合物溶出，因此脱色后再经番红复染，呈红色。

操作步骤：（*仅供参考*）

1. 涂片固定：

准备洁净、干燥的载玻片，菌液涂片固定，固定时通过火焰 3-4 次即可，不可过热，以载玻片不烫手为宜。

2. 染色：

一般包括初染、媒染、脱色、复染等四个步骤，具体操作方法是：

① 滴加结晶紫染色液后，染色 1 分钟，水洗。
② 滴加碘液后染色 1 分钟，水洗。
③ 滴加脱色液，摇动玻片，根据涂片厚度，脱色约 20-60 秒，水洗，吸去水分。
④ 滴加番红染色液后，染色 1 分钟，水洗。
⑤ 滤纸吸干多余水分或在空气中晾干后，100 倍油镜镜检。

革兰氏染色的关键在于严格掌握脱色程度，如脱色过度，则阳性菌可被误染为阴性菌；而脱色不够时，阴性菌可被误染为阳性菌。此外，菌龄也影响染色结果，如阳性菌培养时间过长，或已死亡及部分菌体自行溶解，都常呈阴性反应。

染色结果：

革兰氏阴性菌	红色
革兰氏阳性菌	紫色

以均匀分散开的细菌的革兰氏染色反应为准，过于密集的细菌，常常呈现假阳性。

注意事项：

1. 标本涂片不宜太厚，建议按操作要求进行。若涂片较厚，应延长脱色时间，至不再出现紫色为止。
2. 玻片通过火焰温度不能太高。碘液变透明，则不能使用。
3. 如无油镜观测条件或切片须长期保存，可于晾干后滴加中性树脂封片，40 倍镜观察。
4. 水洗时动作要轻柔，沿载玻片对角线方向用洗瓶冲洗，以免把菌体冲掉。

注：更多使用本产品的文献请参考索莱宝官网。

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Gram Stain Kit

Cat: G1060
Size:4×10mL/4×100mL/4×500mL
Storage: 2-8℃, avoid light, valid for 1 year.

Kit Components

Reagent	4×10mL	4×100mL	4×500mL	Storage
Reagent A: Gram's Crystal Violet Solution	10mL	100mL	500mL	2-8℃
Reagent B: Gram's Safranin Solution	10mL	100mL	500mL	2-8℃
Reagent C: Gram's Iodine Solution	10mL	100mL	500mL	2-8℃, avoid light
Reagent D: Gram's Decolorizing Solution	10mL	100mL	500mL	RT

Introduction

The Gram Stain is a different staining technique most widely applied in microbiology. Gram staining is based on the ability of bacteria cell wall to retaining the crystal violet dye during solvent treatment. The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than gramnegative bacteria. Bacteria cell walls are stained by the Gram's Crystal Violet Solution. Iodine is subsequently added as a mordant to form the crystal violet-iodine complex so that the dye cannot be removed easily. However, subsequent treatment with Gram's Decolorizing Solution dissolves the lipid layer from the gram-negative cells. As a result, the diffusion of the violet-iodine complex is blocked, and the bacteria remain stained. The length of the decolorization is critical in differentiating the gram-positive bacteria from the gram-negative bacteria. Finally, counterstain with Gram's Safranin Solution, the gram-negative bacteria is stained a pink color.

Protocol(*for reference only*)

1. Prepare a bacterial smear on clear, dry glass slide, and fix it through a gentle flame 3-4times while moving the slide in a circular fashion to avoid localized overheating.It is appropriate not to burn your hands.
2. Gram Staining:

1) Flood with Gram's Crystal Violet Solution for 1 min.Wash with distilled water.
2) Flood the smear with Gram’s Iodine Solution for 1 min.Wash with distilled water.
3) Decolorize with Gram's Decolorizing Solution for 20 to 60 s until the blue dye no longer flows.
4) Wash with distilled water.Counterstain with Gram's Safranin Solution for 1 min.Wash with distilled water .
5) Allow the slide to air dry or blot dry between sheets of clean bibulous paper and view under oil immersion lens.

Result

Gram-Positive Organisms	Bluish Purple
Gram-Negative Organisms	Pinkish Red

Note

1. The specimen smear should not be too thick and should be carried out in strict accordance with the operation requirements. If the smear is thick, the decolorization time should be extended until purple no longer appears.
2. The temperature of slide on flame should not be too high.If the iodine solution becomes colorless, change it.
3. If there is no oil microscope observation condition or the slices need to be preserved for a long time, the neutral resin seal can be added after drying and observed under 40 times microscope.
4. When washing, the action should be gentle, and the washing bottle should be used along the diagonal direction of the slide to avoid washing off the bacteria.