V02

结晶紫染色液（0.4%）

货号：G1070
规格：100mL
保存：2-8，避光保存，有效期1年。

产品介绍：

结晶紫染色液（0.4%）是革兰氏染色法中的一种成分，主要由草酸铵、结晶紫、乙醇等组成。革兰阳性菌染色中，细胞经草酸铵结晶紫染色后再经Gram碘液处理，形成不溶性复合物，该复合物不能通过细胞壁，不易被脱色，所以保持紫色。染色后细菌与环境形成鲜明对比，可以清楚地观察到细菌的形态、排列及某些结构特征，而用以分类鉴定。

自备材料：

接种环或挑取细菌的其他工具、酒精灯、载玻片、光学显微镜

操作步骤：(*仅供参考*)

1. 按实验具体要求操作或参考下列革兰氏染色的方法。
2. 涂片：取待检细菌，于载玻片中央涂成薄层或者在载玻片上滴加少许无菌水，取菌与水混合均匀，涂成薄层。
3. 干燥：涂片后在室温下自然干燥，也可在酒精灯上略加温，使之迅速干燥。
4. 固定：手持载玻片一端，标本面朝上，在酒精灯的火焰外侧快速来回移动3-5次，每次1s，温度不宜过高，防止菌体蛋白变性，放置待凉后染色。也可以用甲醇或乙醇固定。。
5. 初染：滴加结晶紫染色液（0.4%）染色1-2min，清水冲洗去染色液。
6. 媒染：滴加Gram碘液，并覆盖载玻片室温放置1-2min，水洗。
7. 脱色：滴加脱色液摇动，摇动10-30s，直至流下的脱色液不出现紫色为止，立即用水冲去脱色液，终止反应。
8. 复染：滴加沙黄染色液染色，30-60s，水洗。
9. 干燥。镜检：置油镜观察。

染色结果：

革兰氏阳性菌	蓝色至紫色
革兰氏阴性菌	红色

注意事项：

1. 涂片之前，应事先在背面做好圆圈标记，以便判断后续试验的位置。
2. 取细菌时，应注意自我防护，拔或塞试管塞时，应将试管口通过火焰略加烧灼，最后将接种环在火焰上烧灼灭菌。
3. 加热固定涂片时，应注意玻片勿太靠近火焰，一般要求玻片温度不超过60℃，以玻片背面触及手背皮肤不觉过烫为宜。
4. 革兰氏染色的关键在于严格掌握脱色程度，脱色时间应根据经验判断。脱色过度，阳性菌可被误染为阴性菌；脱色不够，阴性菌可被误染为阳性菌。
5. 待检细菌培养时间会影响染色，阳性菌培养时间过长或已死亡或细菌溶解，常呈阴性。
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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Crystal Violet Ammonium Oxalate Solution, 0.4%

Cat: G1070
Size: 100mL
Storage: 2-8℃, avoid light, valid for 1 year.

Introdution

Crystal Violet Ammonium Oxalate Solution, 0.4% is a component of Gram's staining method. It is mainly composed of ammonium oxalate, crystal violet and ethanol. In Gram-positive bacteria staining, cells are stained with ammonium oxalate crystal violet and Gram iodine solution to form an insoluble complex. The complex could not pass through the cell wall and could not be destained easily, so it remained purple. After dyeing, the bacteria formed a sharp contrast with the environment, and the morphology, arrangement and some structural characteristics of bacteria could be clearly observed for classification and identification.

Self provided materias

Inoculation ring or other tools for picking up bacteria, Alcohol lamps, Slides, Optical microscope.

Protocol(*for reference only*)

Operate according to the specific requirements of the experiment or refer to the following methods of Gram staining.

1. **Smear:** Take the bacterium to be examined and smear it in the center of the slide or add a little sterile water on the slide. Mix the bacterium with water evenly and smear it in a thin layer.
2. **Drying:** After smear, it can be dried naturally at room temperature or heated slightly on alcohol lamp to make it dry quickly.
3. **Fixation:** Hold the slide with sample facing upward and move 3-5 times quickly outside the flame of alcohol lamp for 1 second each time. The temperature should not be too high to prevent the degeneration of bacterial proteins. Stain after cooling. It can also be fixed with methanol or ethanol.
4. **Primary dyeing:** Add Crystal Violet Ammonium Oxalate Solution, 0.4% to dye for 1-2 min,then rinse the dye solution with water.
5. **Mordant dyeing:** Add Gram iodine solution, and cover the slide at room temperature for 1-2 min, then wash it with water.
6. **Destaining:** Add the destaining solution and shake it for 10-30 s until the destaining solution does not appear purple. Rinse the destaining solution with water immediately to stop the reaction.
7. **Re-dyeing:** Re-dyeing with Safranin stain solution for 30-60 s, then washing with water.
8. **Observation:**View under the Oil immersion microscope after drying.

Result

Gram-positive bacteria	Blue to Purple
Gram-negative bacteria	Red

Note

1. Before smear, make the circle mark on the back in advance to judge the position of subsequent test.
2. When taking bacteria, pay attention to self-protection. When pulling or plugging the test tube plug, we should burn the test tube orifice slightly through the flame, and finally burn the inoculation ring on the flame to sterilize.
3. When heating the fixed smear, pay attention to the slide not too close to the flame. Generally, the temperature of the slide should not over 60℃.
4. The key of Gram staining is to strictly control the destaining degree, and the destaining time should be judged by experience. If destaining is excessive, positive bacteria can be mistakenly stained as negative bacteria; if destaining is insufficient, negative bacteria can be mistakenly stained as positive bacteria.
5. The culture time of the bacteria to be examined will affect the staining. When the culture time of the positive bacteria is too long or the bacteria has died or dissolved, the result is often negative.
6. For your safety and health, please wear experimental clothes and disposable gloves.