V02

改良 Gram-Weigert 革兰染色试剂盒

货号：G3230
规格：4×50mL
保存：室温，避光保存，有效期 6 个月。

产品组成：

名称	4×50mL	保存
试剂 A：苏木素染色液	50mL	2-8℃，避光
试剂 B：伊红染色液	50mL	室温，避光
试剂 C：苯胺结晶紫染色液	50mL	室温，避光
试剂 D：Weigert 碘液	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，亦是一种复染法。通过此法染色可将细菌鉴别为革兰阳性菌和革兰阴性菌两大类。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。在同样染色环境中利用细菌不同的等电点（Gram 阳性细菌等电点为 pH2.0-3.0，Gram 阴性细菌等电点为 pH4.0-5.0），阳性细菌带的负电荷比阴性细菌带的负电荷多，与带正电荷的碱性染料如结晶紫结合较牢，再加入媒染剂（碘）进入菌体后，与染料结合形成不溶于水的结晶紫-碘-蛋白复合物，并与阳性菌菌体内的核糖核酸镁盐结合，使已着色的细菌不易脱色；而分化剂（苯胺、丙酮等）不易透过阳性菌的细胞壁，故阳性菌不易褪色，但分化剂容易进入阴性菌菌体内，溶解染料和碘复合物，使阴性菌脱色。

改良 Gram-Weigert 革兰染色试剂盒是在经典的革兰染色配方进行改进，使用苏木素和 Weigert 碘液加强染色，该法也是在草酸铵结晶紫法演化过来的。临床标本直接涂片，背景干净，胞内吞噬体清晰易辨认，细菌染色特征典型。可以区分 Gram 阳性细菌和阴性细菌，尤其适用于鉴别细菌和非细菌的蓝色微颗粒状物质（如钙盐）。

自备材料：

10%福尔马林固定液，温箱或水浴锅，显微镜。

操作步骤：（*仅供参考*）

1. 组织固定于 10%福尔马林固定液中，常规脱水包埋。
2. 切片厚 4-5μm，常规脱蜡至水。
3. 苏木素染色液染胞核 3-5min，倾去染液，流水冲洗 10min。
4. 入伊红染色液加盖 56℃浸染 5-10min，倾去染液，稍水洗。
5. 苯胺结晶紫染色液滴染于切片上 5min，倾去染液，用滤纸稍吸干切片周围余液。
6. Weigert 碘液滴染于切片上 2-3min，倾去碘液，用滤纸反复吸干切片上的水分。
7. 用苯胺二甲苯溶液（1:2）分化，并不时轻轻摇动，至切片无紫色脱出，立即用二甲苯洗涤，并在镜下观察。
8. 如果分化不够可再次滴加苯胺分化液，直至切片上革兰阳性菌显示清楚为止。
9. 滴入新的二甲苯反复洗涤多次，彻底把苯胺洗去，中性树胶封固。

染色结果：

革兰阳性细菌和纤维素	蓝紫色
革兰阴性细菌	红色
细胞核	蓝色
其他	淡红色

注意事项：

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1. 经 Weigert 碘液和水洗后，必须用滤纸反复吸干切片上的水分，再滴加苯胺二甲苯溶液（1:2），否则可能导致分化不均匀。
2. 经二甲苯冲洗后，应在镜下观察。如分化不足，可再滴入苯胺二甲苯溶液（1:2）继续分化，至阳性菌清晰为止，但注意不要分化过度。
3. 最后用二甲苯反复洗涤切片，把苯胺彻底清除，切片若残留少量苯胺，以后就容易褪色。
4. 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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Modified Weigert-Gram Stain Kit

V02

Cat: G3230
Size: 4×50mL
Storage: RT, avoid light, valid for 6 months.

Kit components

Reagent	4×50mL	Storage
Reagent A：Hematoxylin Solution	50mL	2-8℃, avoid light
Reagent B：Eosin Solution	50mL	RT, avoid light
Reagent C：Aniline Crystal Violet Solution	50mL	RT, avoid light
Reagent D：Weigert Iodine Solution	50mL	RT, avoid light

Introduction

Gram staining was invented by Danish doctor Christian Gram in 1884. It is not only a differential staining method widely used in bacteriology, but also a double staining method. The bacteria can be identified as Gram-positive bacteria and Gram-negative bacteria by this staining method. The different color reaction of bacteria is due to the difference of cell wall permeability to ethanol and anti decolorization ability, which is mainly determined by the thickness and structure of peptidoglycan layer. In the same dyeing environment, using different isoelectric points of bacteria (the isoelectric points of gram positive bacteria are pH 2.0-3.0, and the isoelectric points of gram negative bacteria are pH 4.0-5.0), the negative charge of positive bacteria is more than that of negative bacteria, which is firmly combined with positively charged alkaline dyes such as crystal violet. After adding mordant (iodine) into the bacteria, It combines with dyes to form water-insoluble crystal violet iodine protein complex, and combines with ribonucleic acid magnesium salt in positive bacteria, so that colored bacteria are not easy to decolorize; The differentiation solution(aniline, acetone, etc.) is not easy to penetrate the cell wall of the positive bacteria, so the positive bacteria are not easy to fade, but the differentiation agent is easy to enter the negative bacteria to dissolve the dye and iodine complex to decolorize the negative bacteria.

The Modified Weigert-Gram Stain Kit is improved on the classical gram dyeing formula, and the hematoxylin and weigert iodine solution are used to strengthen the dyeing. This method is also evolved from the ammonium oxalate crystal violet method. The clinical specimens can be smeared directly, the background is clean, the intracellular phagocytes are clear and easy to identify, and the bacterial staining characteristics are typical. Gram positive bacteria and gram negative bacteria can be distinguished, especially suitable for identifying bacterial and non bacterial blue micro granular substances (such as calcium salt).

Self Provided Materials

10% formalin fixative, incubator or water bath, optical microscope.

Protocols*(for reference only)*

1. Fix the tissue in 10% formalin fixative, dehydrate and embed by routine method.
2. Cut slice in thickness of 4-5μm. Conventionally dewax to water.
3. Stain the nucleus with Hematoxylin Solution for 3-5min, and wash with running water for 10min.
4. Soak in Eosin Solution and cover stain at 56 ℃ for 5-10min, then wash it with water.
5. Drip Aniline Crystal Violet Solution onto the slice and stain for 5min, remove the staining solution and slightly suck dry the remaining solution around the slice with filter paper.
6. Drip Weigert Iodine Solution onto the slice for 2-3min, remove the iodine solution and repeatedly suck dry the water on the slice with filter paper.
7. Decolorize with aniline xylene solution (1:2), shake gently from time to time until there is no purple prolapse on the slice, wash with xylene immediately and observe under the microscope.
8. If the differentiation is not enough, add aniline differentiation solution again until the Gram-positive bacteria are clearly displayed on the section.
9. Drop in new xylene, wash repeatedly for many times, wash away aniline thoroughly, and seal with resinene.

Result

Gram positive bacteria and cellulose	Blue Purple
Gram negative bacteria	Red

Nucleus	Blue
Background	Light Red

Note

1. After washing with Weigert Iodine Solution and water, must repeatedly suck up the water on the slice with the filter paper , and then add aniline xylene solution (1:2), otherwise it may lead to uneven differentiation.
2. After washing with xylene, shall observe under the microscope. If the differentiation is insufficient, drop aniline xylene solution (1:2) to continue differentiation until the positive bacteria are clear, but be careful not to over differentiate.
3. Finally, wash the slice repeatedly with xylene to completely remove the aniline. If a small amount of aniline remains in the slices, it is easy to fade in the future.
4. For your safety and health, please wear experimental clothes and disposable gloves.

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