V02

Conn 改良 Weigert 革兰染色试剂盒

货号：G3220
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)：胭脂红染色液	50mL	室温，避光
试剂(B)：酸性乙醇分化液	50mL	室温
试剂(C)：草酸铵结晶紫染色液	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(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。经结晶紫染色的细胞用碘液处理后形成不溶性复合物，乙醇能使它脱色。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，故阳性菌不易退色；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。

Conn 改良 Weigert 革兰染色试剂盒是在经典的革兰染色配方进行改进，使用胭脂红作为衬染剂，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、显微镜

操作步骤：（*仅供参考*）

1. 组织固定于 10%福尔马林中，常规脱水包埋。
2. 切片厚 4μm，常规脱蜡至水。
3. 胭脂红染色液滴染切片 5min，倾去染液。
4. 酸性乙醇分化液分化 2-5s。
5. 蒸馏水冲洗 3-5s。
6. 草酸铵结晶紫染色液滴染 2min，倾去染液。用滤纸稍吸干切片周围余液。
7. Weigert 碘液处理切片 2-5min，倾去碘液。蒸馏水冲洗后晾干切片。
8. 95%乙醇分化 2-5min 至切片无紫色脱出，立即用无水乙醇洗涤 1-2 次终止分化。
9. 二甲苯透明 2 次，每次 1min，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	红色

注意事项：

1. 切片在胭脂红染色液中染色后不用水洗，直接滴入酸性乙醇进行分化并固定着染胭脂红的颜色。

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

2. 用 95%乙醇分化时，可轻轻摇动切片使分化均匀。如分化慢时可更换新的 95%乙醇。至切片无紫色脱出，立即倾去 95%乙醇，使用无水乙醇清洗终止分化。
3. 经无水乙醇冲洗后，应在镜下观察。如分化不足，可再滴入 95%乙醇继续分化，至 Gram 阳性菌清晰为止，但注意不要分化过度。
4. Gram 染色阳性的细菌必须具有未受损的细菌壁，如细菌壁受破损，则染色呈阴性。
5. 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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Weigert's Gram Stain Kit(Conn Modified)

Cat: G3220
Size: 4×50mL
Storage: RT, avoid light, valid for 6 months.

Kit components

Reagent	4×50mL	Storage
Reagent(A): Carmine Staining Solution	50mL	RT, avoid light
Reagent(B): Acid Alcohol Differentiation Solution	50mL	RT
Reagent(C): Ammonium Oxalate Crystal Violet Staining 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.

Weigert's Gram Stain Kit(Conn Modified) is improved from the classical gram dyeing formula, using carmine as the lining agent. 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, 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. Drip Carmine Staining Solution onto the section and stain for 5min, then remove the solution.
4. Treat with Acid Alcohol Differentiation Solution for 2-5s.
5. Rinse with distilled water for 3-5s.
6. Drip dyeing with Ammonium Oxalate Crystal Violet Staining Solution for 2 min, and remove the dyeing solution.Slightly suck dry the remaining solution around the slice with filter paper.
7. Drip Weigert Iodine Solution onto the slice for 2-5 min, rinse with distilled water and air dry the slices.
8. Decolorize with 95% ethanol for 2-5min, shake gently from time to time until there is no purple prolapse on the slice, wash with 100% ethanol immediately for 1-2 times to stop differentiation.
9. Transparent by xylene for twice, each for 1min, then seal with resinene.

Result

Gram positive bacteria and cellulose	Blue Purple
Gram negative bacteria	Red
Nucleus	Red

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Note

1. After staining with Carmine Staining Solution, without washing, directly differentiate with Acid Alcohol Differentiation Solution to avoid fading.
2. When differentiating with 95% ethanol, gently shake the slices to make the differentiation uniform. If the differentiation is slow, can replace new 95% ethanol . Until there is no purple solution out of the slice, immediately pour 95% ethanol and wash with absolute ethanol to stop differentiation.
3. After washing with absolute ethanol, shall observe under the microscope. In case of insufficient differentiation, 95% ethanol can be added to continue differentiation until Gram-positive bacteria are clear, but be careful not to over differentiate.
4. Gram positive bacteria must have an undamaged bacterial wall. If the bacterial wall is damaged, the staining is negative.
5. For your safety and health, please wear experimental clothes and disposable gloves.