V02

鞭毛染色试剂盒(银染法)

货号：G1136
规格：2×100mL
保存：2-8℃，避光保存，有效期 6 个月。

产品组成：

名称		2×100mL	保存
试剂(A): 鞣酸染色液	A1: 鞣酸稀释液	50mL	室温
	A2: 鞣酸溶液	50mL	室温，避光
临用前取 A1、A2 等量混合，即为鞣酸染色液			
试剂(B): 氨银染色液		100mL	2-8℃，避光

产品介绍：

细菌鞭毛是细菌的运动器官，幽门螺杆菌能够从强酸性的胃内腔穿过胃上皮细胞上的黏液层达到胃上皮细胞的中性环境，这就是鞭毛运动作用的很好例证。通过鞭毛染色，可以观察到鞭毛形态、数量和鞭毛在菌体分布的位置，鞭毛数量和在菌体上的分布位置是鉴定细菌的重要依据之一。

鞭毛染色试剂盒(银染法)采用镀银染色法，该染色法的优点是采用氨银染色液作为核心染料，试剂比较灵敏，操作简单，结果判断更可靠。

自备材料：

酒精灯，载玻片，蒸馏水，接种环，显微镜

操作步骤：（*仅供参考*）

1. 在洁净无油脂的载玻片上滴加 2 滴蒸馏水。
2. 用接种环挑取无菌蒸馏水，再与血平板上菌落接触，允许细菌游到接种环蒸馏水中，再将接种环移到玻片上蒸馏水顶部轻点 2 次。
3. 轻轻摇动玻片，使细菌分布均匀。切勿研磨和搅动，以防鞭毛脱落。
4. 置室温或 37℃恒温箱内干燥固定。
5. 取刚配制的鞣酸染色液加入干净容器或者试管中，充分混匀，用酒精灯缓慢加热，稍微冷却，立即进行染色。
6. 滴加经加热处理的鞣酸染色液于载玻片上，染色 30-40s，蒸馏水缓慢冲洗，甩干。
7. 滴加氨银染色液，小火焰加热至冒气泡，蒸馏水缓慢冲洗，自然干燥。
8. 镜检：从涂片边缘开始，由外及里，逐渐移至中心。细菌分布少的地方，鞭毛容易观察。细菌密集的地方，鞭毛被菌体挡住，不易观察。

染色结果：

菌体	深褐色
鞭毛	褐色

注意事项：

1. 玻片应洁净，无油污。
2. 染色的菌种应连续传代多次，处于生长活跃期。
3. 染色过程应小心操作，防止鞭毛脱落。 固定时不宜用高热火焰固定。
4. 配制好的鞣酸染色液不宜久置，尽量在 10min 内使用。
5. 氨银染色液不稳定，应严格 4℃避光保存，出现严重浑浊时应弃用。
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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Flagella Stain Kit(Silver Method)

Cat: G1136
Size: 2×100mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		2×100mL	Storage
Reagent(A): Tannic Acid Working Solution	A1: Tannic Acid Diluent	50mL	RT
	A2: Tannic Acid Solution	50mL	RT, avoid light
Mix A1and A2 with the radio of 1:1 to form Tannic Acid Working Solution before use.			
Reagent(B):Ammonia Silver Staining Solution		100mL	2-8℃, avoid light

Introduction

Bacterial flagellum is the motor organ of bacteria.Helicobacter pylori can pass through the mucus layer of gastric epithelial cells from the strongly acidic gastric cavity to the neutral environment of gastric epithelial cells, which is a good example of flagellum movement. Flagella morphology, quantity and location of flagella in bacteria can be observed by flagella staining. Flagellum quantity and its distribution on bacteria are one of the important bases for identifying bacteria.

This kit use silver staining method. The advantage of the method is that Ammonia Silver Staining Solution is used as the core dye. The reagent is more sensitive, the operation is simple and the result is more reliable.

Self Provided Materials

Alcohol lamp, slide, distilled water, inoculation ring, microscope.

Protocol(*for reference only*)

1. Drop 2 drops of distilled water on a clean, fat-free slide.
2. Select the sterile distilled water with the inoculation ring, then contact the colony on the blood plate, allow the bacteria to swim into the inoculation ring distilled water, and then move the inoculation ring to the top of the distilled water on the slide twice.
3. Shake the slide to distribute the bacteria. Do not grind and stir with the slide to avoid flagella falling off.
4. Dry in a room temperature or 37℃ incubator (not fixed by flame).
5. Add the Tannic Acid Working Solution to a clean container or test tube, mix it well, heat it slowly with an alcohol lamp, cool it slightly, and dye it immediately.
6. Drop the heated Tannic Acid Working Solution onto the slide and dye it for 30-40 s. Rinse it slowly with distilled water and shake it dry.
7. Drop Ammonia Silver Staining Solution, heat in small flame until bubbling, rinse with distilled water, and dry naturally.
8. Microscopic examination should begin at the edge of the smear and gradually move to the center from the outside to the inside. Where bacteria are less distributed, flagellum is easy to observe. Where bacteria are concentrated, flagellum is blocked by fungi and is not easy to observe.

Result

Thalli	Dark Brown
Flagellum	Brown

Note

1. Slides should be clean and free from oil pollution.
2. The bacteria sample for staining must be in the active stage of growth，which should be successive subculture for many times.
3. Operate carefully in the dyeing process to prevent flagella falling off.It is not appropriate to fix with high-temperature flame.
4. The prepared Tannic Acid Working Solution should be used within 10 min as far as possible .
5. Ammonia Silver Staining Solution is unstable. It should be stored avoiding light at 4℃ strictly and should be discarded when serious turbidity occurs.
6. For your safety and health, please wear experimental clothes and disposable gloves.

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