

螺旋体菌染色试剂盒(Levaditi 法)

货号: G3090

规格: 3×50mL

保存: 2-8℃, 避光保存, 有效期 3 个月。

产品组成:

名称		3×50mL	保存
试剂(A):硝酸银溶液		2×50mL	2-8℃, 避光
试剂(B):Levaditi 还原液	B1:Levaditi 还原液 A	25mL	室温
	B2:Levaditi 还原液 B	25mL	室温, 避光
临用前, 按 B1:B2=1:1 混合, 即为 Levaditi 还原液, 即配即用。			

产品说明:

螺旋体(spirochaeta)是一类细长、弯曲呈螺旋状、运动活泼的原核细胞型微生物,具有细菌的基本结构,细胞壁有脂多糖和壁酸。螺旋体分为密螺旋体(如梅毒螺旋体)、疏螺旋体(如回归热螺旋体)、钩端螺旋体等。梅毒螺旋体有 8~12 均匀的螺旋,两端尖直;钩端螺旋体细长,数目较多而且细密,一端或两端弯曲呈钩状,菌体亦常屈曲呈 C 形或 S 形。梅毒螺旋体和钩端螺旋体可用镀银法进行显色,如 Levaditi 法、Warthin-Starry 法。

螺旋体菌染色试剂盒(Levaditi 法)是利用螺旋体在特定条件下可从银液中吸附银离子,经还原液处理后,吸附的银离子被还原为黑色的金属银而显色。该染色液主要用于显示梅毒螺旋体,亦可显示钩端螺旋体,但较少显示热回归螺旋体,对肝脏梅毒树胶肿和肝脏钩端螺旋体具有鉴别作用。

操作步骤: (仅供参考)

1. 组织切片厚度 1-2mm 为佳,置于 10%福尔马林中固定 2-4 天。
2. 流水冲洗过夜。
3. 95%乙醇浸泡 24h。
4. 蒸馏水浸洗并不停摇动,直至组织下沉为止。
5. 更换新的蒸馏水浸洗 10min。
6. 入硝酸银溶液,加盖置于 37℃恒温箱孵育 1 天,更换新的硝酸银溶液,继续 37℃恒温箱孵育 2 天。
7. 蒸馏水浸洗 3 次,每次 10min。
8. 入配制好的 Levaditi 还原液,加盖室温避光孵育 2 天。
9. 蒸馏水浸洗 3 次,每次 2min。
10. 常规脱水透明,浸蜡包埋。
11. 切片厚度 6-8μm,贴片,烤干。
12. 二甲苯脱蜡 2 次,中性树胶封固。

染色结果:

梅毒螺旋体、钩端螺旋体	黑色或棕黑色
背景	淡黄至棕黄色

注意事项:

1. 实验所用器皿需用硫酸洗液浸泡，洗净。
2. 步骤 4、5 的洗片步骤很有必要，切记要把组织中的甲醛和乙醇彻底清除，否则易导致组织内非特异的银盐沉淀。
3. 某些细菌和真菌菌丝也会被该染液染成黑色，应注意螺线体为螺旋状形态，并加以鉴别。
4. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Spirochete Staining Kit (Levaditi Method)

Cat: G3090

Size: 3×50mL

Storage: 2-8°C, avoid light, valid for 3 months.

Kit Components

Reagent		3×50mL	Storage
Reagent(A): Silver Nitrate Solution		2×50mL	2-8°C, avoid light
Reagent(B):Levaditi Solution	B1:Levaditi Solution A	25mL	RT
	B2:Levaditi Solution B	25mL	RT, avoid light
Before use, mix B1 with B2 in equal amount to prepare LevaditiSolution. It is ready to use.			

Introduction

Spirochaeta is a kind of slender, spiral and moving prokaryotic microorganisms. It has the basic structure of bacteria, and the cell wall has lipopolysaccharide and wall acid. Spirochetes are divided into dense spirochetes (such as *Treponema pallidum*), *Borrelia* (such as regressive heat spirochete), *Leptospira*, etc. *Treponema pallidum* has 8 ~ 12 uniform spirals with straight ends; *Leptospira* are slender, numerous and dense. One or both ends are bent in hook shape, and the bacteria often bend in C-shape or S-shape. *Treponema pallidum* and *Leptospira* can be colored by silver plating, such as Levaditi method and Warthin starry method.

Spirochete Staining Kit (Levaditi Method) uses *Leptospira* to adsorb silver ions from silver solution under specific conditions. After treatment with reduction solution, the adsorbed silver ions are reduced to black metal silver for color development. The staining solution is mainly used to display *Treponema pallidum* and *Leptospira*, but less heat regression spirochete. It can distinguish between hepatic syphilis gumma and hepatic *Leptospira*.

Protocol(for reference only)

1. The best thickness of tissue section is 1-2mm. Fix in 10% formalin for 2-4 days.
2. Rinse with running water overnight.
3. Soak in 95% ethanol for 24 hours.
4. Soak in distilled water and shake continuously until the tissue sinks.
5. Replace with new distilled water and soak for 10min.
6. Add Silver Nitrate Solution, cover and incubate in 37 °C incubator for 1 day, replace with new Silver Nitrate Solution and continue to incubate in 37 °C incubator for 2 days.
7. Soak with distilled water 3 times, each time for 10min.
8. Add the prepared Levaditi Solution, cover it at room temperature and incubate it in the dark for 2 days.
9. Soak with distilled water for 3 times, each time for 2min.
10. Transparent by series of ethanol and embed in wax.
11. Cut slice in 6-8 μm thickness. Patch and dry.
12. Dewaxi by xylene twice and seal with resinene.

Result

<i>Treponema pallidum</i> , <i>Leptospira</i>	Black or Brownish Black
Background	Light yellow to brownish yellow

Note

1. The utensils used in the experiment should be soaked in sulfuric acid lotion and washed clean.
2. The film development steps in steps 4 and 5 are very necessary. Remember to completely remove the formaldehyde and ethanol in the tissue, otherwise it is easy to cause the precipitation of silver salt in the tissue.
3. Some bacterial and fungal hyphae will also be dyed black by the dye solution. It should be noted that the spirochete is spiral and identified.
4. For your safety and health, please wear experimental clothes and disposable gloves.