V02

网状纤维染色试剂盒(改良 Gordon-Sweets 法)

货号：G3525
规格：6×50mL
保存：2-8℃，避光保存，有效期 6 个月。

产品组成：

名称		6×50mL	保存
试剂(A): Gordon- Sweets 氧化剂	A1: GS 氧化剂 A	25mL	室温，避光
	A2: GS 氧化剂 B	25mL	室温
临用前，取 A1、A2 等量混合即为 Gordon-Sweets 氧化剂，即配即用。			
试剂(B): 草酸溶液		50mL	室温
试剂(C): 硫酸铁铵溶液		50mL	室温，避光
试剂(D): Gordon-Sweets 银氨溶液		50mL	2-8℃，避光
试剂(E): Gordon-Sweets 还原剂		50mL	室温
试剂(F): 核固红染色液		50mL	室温，避光

产品介绍：

网状纤维(Reticular fiber)是网状结缔组织内的一种纤维，由网状细胞所产生，直径多在 0.2～1.0μm，有韧性而没有弹性。网状纤维的染色方法很多，但染色原理基本一致，大都采用氨银浸法。本改良法省略传统方法中调色和还原的步骤，使网状纤维对比更清晰。

网状纤维染色试剂盒(改良 Gordon-Sweets 法)采用酸性氧化剂和核固红复染液使结果更清晰。冰冻切片、低温切片和火棉胶切片均可用于网状纤维染色。

操作步骤：（*仅供参考*）

试剂(F)：核固红染色液可能会由于絮凝产生悬浮物或少量沉淀，建议取上清使用或沸水浴 5-10min 后晾至 30-40℃使用。（见注意事项 2）

1. 组织固定于 10%福尔马林固定液，常规脱水包埋。切片厚 4μm，常规脱蜡至水。
2. 切片滴加配制好的 Gordon-Sweets 氧化剂氧化 3min，蒸馏水洗 10s。
3. 滴加草酸溶液漂白 1min，蒸馏水洗 2 次，每次 1min。
4. 滴加硫酸铁铵溶液媒染 10min，蒸馏水洗 2 次，每次 1min。
5. 滴加 Gordon-Sweets 银氨溶液染色 11s，蒸馏水洗 2 次，每次 2min。
6. 滴加 Gordon-Sweets 还原剂还原 2min，蒸馏水冲洗 1min。
7. 滴加核固红染色液复染细胞核 10min，蒸馏水洗 20s。
8. 常规脱水透明，中性树胶封片。

染色结果：

网状纤维	黑色
胶原纤维	黄色至黄棕色
细胞核	红色
细胞质	淡黄色

注意事项：

1. 玻璃器皿必须用洗涤液浸泡 1 天，自来水冲洗干净，蒸馏水冲洗 2 次。
2. 试剂(F)：核固红染色液为胶体性质溶液，低温（低于 25℃）保存或长期储存由于絮凝产生悬浮物或少量沉淀，属于正常现象，一般不影响使用。如移液器吸取观察到明显浑浊，可拧紧瓶盖沸水浴 5-10min 重新制备分散均匀的胶体溶液来恢复使用。
3. 推荐使用 10%福尔马林固定液固定，不宜采用含汞的固定剂如 Zenker 液，否则易导致非特异性沉淀。
4. Gordon-Sweets 氨银溶液不太稳定，对光的敏感性强，应 4℃避光保存，恢复至室温后使用。
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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Reticulin Stain Kit(Gordon-Sweet Sliver Method)

Cat: G3525
Size: 6×50mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent		6×50mL	Storage
Reagent(A):Gordon-Sweets Oxiadant	A1: GS Oxiadant A	25mL	RT, avoid light
	A2: GS Oxiadant B	25mL	RT
Mix equal parts of GS Oxidant A and B to form Gordon-Sweets Oxiadant before use. It is ready to use.			
Reagent(B): Oxalic Acid Solution		50mL	RT
Reagent(C): Ferric Ammonium Sulfate Solution		50mL	RT, avoid light
Reagent(D):Gordon-Sweets Ammoniacal Silver Solution		50mL	2-8℃, avoid light
Reagent(E): Gordon-Sweets Reductant		50mL	RT
Reagent(F): Nuclear Fast Red Solution		50mL	RT, avoid light

Introduction

Reticular fiber is a kind of fiber in reticular connective tissue, which is produced by reticular cells. Its diameter is mostly 0.2-1.0μm. it has toughness but no elasticity. There are many dyeing methods for reticular fiber, but the dyeing principle is basically the same, and most of them adopt ammonia silver staining method. In the traditional method, after reduction, gold chloride solution is used for color matching, and then hypo solution is used to wash away the unreduced silver salt on the tissue. This improved method omits this step and makes the contrast of reticular fiber clearer.

Reticulin Stain Kit(Gordon-Sweet Sliver Method) uses acid oxidant and nuclear fast red re-dyeing solution to get better result. Frozen section, low temperature section and collodion section can be used for reticular fiber staining.

Protocol(*for reference only*)

Reagent(F): Nuclear Fast Red Solution may produce suspended solids or a small amount of precipitation due to flocculation. It is recommended to take supernatant or boil water bath for 5-10min and then air it to 30-40 ℃. (see Note 2)

1. Fix the tissue in 10% formalin fixative, then conventionally dehydrate and embed.
2. Cut into paraffin section in 4μm thick, and dewax to distilled water.
3. Drop in the prepared Gordon-Sweets Oxiadant and oxidize for 3min.Rinse with running water for 20s.
4. Add Oxalic Acid Solution for 1min,wash with distilled water twice for 1min each.
5. Add Ferric Ammonium Sulfate Solution for 10min,wash with distilled water twice for 1min each.
6. Add Gordon-Sweets Ammoniacal Silver Solution for 11s,wash with distilled water twice for 2min each.
7. Add Gordon-Sweets Reductant for 2min and rinse in running water for 1min.
8. Add Nuclear Fast Red Solution for 10min and wash slightly with water for 20s.
9. Conventionally dehydrate and transparent, seal with resinene.

Result

Reticular Fiber	Black
Collagen Fiber	Yellow to Yellowish Brown
Nucleus	Red
Cytoplasm	Light Yellow

Note

1. Glass container must be soaked in washing solution for one day, washed with tap water and washed twice with distilled water.
2. Reagent(F): Nuclear Fast Red Solution is a colloidal solution, which is stored at low temperature (lower than 25 ℃) or stored for a long time. Suspended solids or a small amount of precipitation are generated due to flocculation, which is a normal phenomenon and generally does not affect the use. If the colloid solution is evenly dispersed in the boiling bath, tighten the bottle cap for 5-10min to recover the turbid solution.
3. 10% formalin fixative is a suitable fixative. It is not suitable to use fixative containing mercury such as Zenker's Solution, otherwise it is easy to cause nonspecific deposition of sections.
4. Gordon-Sweets Ammoniacal Silver Solution should be kept away from light at 4 ℃ and used in RT.
5. For your safety and health, please wear experimental clothes and disposable gloves.