改良神经镀银染色试剂盒(Bielschowsky 法)

货号： G3260
规格： 5×50mL
保存： 2-8℃, 避光保存, 有效期1年。

产品组成：

名称	5×50mL	保存
试剂(A): Bielschowsky银溶液	50mL	2-8℃，避光
试剂(B): 还原剂	50mL	室温
试剂(C): Bielschowsky氨银溶液	50mL	2-8℃，避光
试剂(D): 氯化金溶液	50mL	2-8℃，避光
试剂(E): 海波溶液	50mL	室温

产品介绍：

神经元(Neuron)又称神经细胞，是构成神经系统结构和功能的基本单位。神经元具有长突触(轴突)的细胞，它由细胞体和细胞突起构成。在长的轴突上套有一层鞘组成神经纤维，它的末端的细小分支叫做神经末梢。神经元及神经纤维的染色方法比较多，主要采用镀银染色、焦油紫染色等。

改良神经镀银染色试剂盒(Bielschowsky 法)是典型的镀银染色法，其基本原理为固定后的组织和切片浸染于银溶液中，再用还原剂处理，使银颗粒沉着在轴索的轴浆中使之呈现深棕色或黑色。镀银后可在神经元胞浆内看到许多交错成网的细丝，并伸向树突及轴突中。Bielschowsky 染色常用于诊断和鉴别某些神经系统肿瘤方面。此染色法显示神经纤维瘤、节细胞性神经纤维瘤为阳性，而神经鞘瘤等为阴性。

操作步骤： *（仅供参考）*

还原剂为储备液，使用前用去离子水按 1：3 稀释 4 倍制成还原剂工作液使用。（见注意事项 3）

1. 石蜡切片切 8~15μm，脱蜡至水。
2. 滴加 Bielschowsky 银溶液覆盖切片，并置于 37℃温箱内避光浸染 25~35 min。蒸馏水洗 2～3min。
3. 滴加还原剂工作液覆盖切片还原 5~10 秒，至切片略呈黄色。蒸馏水洗 3~5 min。（见注意事项 4）
4. 滴加 Bielschowsky 氨银溶液覆盖切片染色 1~2min。
5. 补加等量还原剂工作液与氨银溶液在切片上快速混匀，避光孵育 5~15min。
6. 倒掉后再次滴加还原剂工作液还原 1min，使切片呈棕黄色为止。（见注意事项 5）
7. 蒸馏水洗 3~5min，滴加海波溶液处理 3~5min，蒸馏水洗 1~2min。
8. 用氯化金溶液调色 3~5min。水洗 3~5min，然后用滤纸吸干多余水分。
9. 95%乙醇及无水乙醇脱水，二甲苯透明，中性树胶封固。

染色结果：

神经元、轴突、神经纤维	深紫色至黑色

注意事项：

1. 所用的玻璃器皿要很清洁，反复用水冲洗及蒸馏水洗。
2. 浸银染色中切片注意要展平避免褶皱，以免着色不匀。
3. 还原剂为储备液，直接使用会导致银非特异不均匀的背景附着，建议用去离子水稀释 4 倍制成工作液使用，也可根据实际实验需求稀释 2-200 倍使用。
4. 冰冻切片可观察到切片变黄，石蜡切片可能没有明显变色反应，属于正常现象。
5. 在氨银溶液上直接滴加还原剂能在一定程度上避免非特异着色。正常情况神经纤维呈铜红色至黑色，无明显颗粒沉积。切片染完后，裱片时要轻拿轻放，以免切片弄碎。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Modified Bielschowsky's Stain Kit

Cat: G3260
Size: 5×50mL
Storage: 2-8℃, avoid light, valid for 1 year.

Kit Components

Reagent	5×50mL	Storage
Reagent(A): Bielschowsky Silver Solution	50mL	2-8℃, avoid light
Reagent(B): Reductant	50mL	RT
Reagent(C): Bielschowsky Ammoniac Silver Solution	50mL	2-8℃, avoid light
Reagent(D): Gold Chloride Solution	50mL	2-8℃, avoid light
Reagent(E): Hypo Solution	50mL	RT

Introduction

Neuron, also known as nerve cell, is the basic unit of the structure and function of nervous system. A cell with a long synapse (axon) that consists of a cell body and processes. The long axon is sheathed with a sheath to form nerve fibers, and the small branches at its end are called nerve endings. There are many staining methods for neurons and nerve fibers, such as silver staining and cresyl violet staining.

Modified Bielschowsky's Stain Kit is a typical silver staining method. Its basic principle is that the fixed tissue and sections are immersed in the silver solution, and then treated with reductant, so that the silver particles settle in the axonal plasma and appear dark brown or black. After silver staining, many interlaced filaments can be seen in the cytoplasm of neurons, and extend to dendrites and axons. The staining method shows that neurofibroma and ganglioneurofibroma are positive, while schwannoma is negative.

Protocols*(for reference only)*

The Reductant is stock solution. Before use, dilute 4 times with distilled water to form Reductant Working Solution.（See Note 3）

1. Cut paraffin section in 8-15μm thick, dewax to distilled water.
2. Add the section in Bielschowsky Silver Solution, and incubate in a 37℃ incubator for 25-35min in dark.Rinse in distilled water for 2-3min.
3. Reduce with Reductant Working Solution for several seconds until the section turns yellow.（See Note 4）
4. Rinse in distilled water for 3-5min.
5. Drop Bielschowsky Ammoniac Silver Solution onto the section and stain for 1-2min.
6. Add an equal amount of reducing agent working solution and ammonia silver solution to quickly mix on the slice, and incubate in dark for 5-15 min.
7. After pouring it out, add the reducing agent working solution again to reduce for 1 minute until the slices turn brownish yellow. (See Note 5)
8. Treat with Hypo Solution for 3-5min.Rinse in distilled water for 1-2min.
9. Match color with Gold Chloride Solution for 3-5min.
10. Rinse in distilled water for 3-5min, then use filter paper to absorb the moisture around the section.
11. Dehydrate in 95% ethanol and absolute ethanol, transparent by xylene and seal with resinene.

Result

Neurons, Axons, Nerve Fibers	Dark Purple to Black

Note

1. The glass container used should be very clean. Wash repeatedly with water and distilled water.
2. In silver staining, the sections should be flattened to avoid wrinkles, so as to avoid uneven coloring.
3. The reducing agent is a reserve solution, and direct use will cause non specific and uneven background adhesion of silver. It is recommended to dilute it 4 times with deionized water to make a working solution for use, or dilute it 2 to 200 times according to actual experimental needs.
4. Frozen sections can be observed to turn yellow, while paraffin sections may not have significant discoloration reactions, which is a normal phenomenon.
5. Directly adding a reducing agent to the silver ammonia solution can to some extent avoid non-specific coloring. Under normal circumstances, the nerve fibers are copper red to black in color, with no obvious particle deposition. After dyeing the slices, it is important to handle them gently when mounting them to avoid crushing them.
6. For your safety and health, please wear laboratory clothes and disposable gloves for operation.