V02

# 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):预染液 | 50mL | 2-8℃，避光 |
| 试剂(B):显色液 | 50mL | 室温 |
| 试剂(C):氨银溶液 | 50mL | 2-8℃，避光 |
| 试剂(D):调色液 | 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。神经元由细胞体和突起构成，突起又可以分为轴突和树突。有髓神经纤维在轴突上套有一层鞘称之为髓鞘，它末端的细小分支叫做神经末梢，神经末梢可与靶肌肉形成运动终板。神经元及神经纤维的染色方法比较多，主要包括镀银染色、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，再用显色液处理，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 法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）*

***显色液为储备液，使用前用去离子水按 1：3 稀释 4 倍制成显色液工作液使用。（见注意事项 3）***

1. 石蜡切片切 8~15μm，脱蜡至水。
2. 滴加预染液覆盖切片，并置于 37℃温箱内避光浸染 25~35 min。蒸馏水洗 2～3min。
3. 滴加显色液工作液覆盖切片还原 10~20 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. 补加等量显色液工作液与氨银溶液在切片上快速混匀，避光孵育 2~10min。
6. 倒掉后再次滴加显色液工作液还原 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. 用调色液调色 1~5min。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. 显色液为储备液，直接使用容易导致非特异着色影响观察，建议用去离子水稀释 4 倍制成工作液使用。
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显色液能在一定程度上提升染色特异性。此时神经纤维应呈棕黄色至黑色，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。



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# Modified Nerve Silver Plating Staining Kit,Bielschowsky's Method

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

## Kit Components

| Reagent | 5×50mL | Storage |
|---|---|---|
| Reagent(A): Pretreatment Solution | 50mL | 2-8℃, avoid light |
| Reagent(B): Chromogenic Solution | 50mL | RT |
| Reagent(C): Ammoniac Silver Solution | 50mL | 2-8℃, avoid light |
| Reagent(D): Toner Solution | 50mL | 2-8℃, avoid light |
| Reagent(E): Hypo Solution | 50mL | RT |

## Introduction

Neurons , also known as nerve cells, are the basic units that make up the structure and function of the nervous system. A neuron consists of a cell body and a protrusion, which can be further divided into axons and dendrites. Myelinated nerve fibers have a sheath over the axon called myelin, and the tiny branches at the end of it are called nerve endings, which can form motor endplates with target muscles. 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 Chromogenic solution is stock solution. Before use, dilute 4 times with distilled water to form Chromogenic Working Solution.（See Note 3）***

1. Cut paraffin section in 8-15μm thick, dewax to distilled water.
2. Add the section in Pretreatment Solution, and incubate in a 37℃ incubator for 25-35min in dark.Rinse in distilled water for 2-3min.
3. Reduce with Chromogenic Working Solution for 10-20s until the section turns yellow.（See Note 4）
4. Rinse in distilled water for 3-5min.
5. Drop Ammoniac Silver Solution onto the section and stain for 1-2min.
6. Add equal amount of Chromogenic Working Solution on the slice, and incubate in dark for 2-10 min.
7. After pouring,add the Chromogenic Working Solution again to reduce for 1 min and fully react. (See Note 5)
8. Treat with Hypo Solution for 3-5min.Rinse in distilled water for 1-2min.
9. Match color with Toner Solution for 1-5min.
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 Chromogenic Solution is a reserve solution, and direct use will cause non specific and uneven background adhesion. It is recommended to dilute it 4 times with distilled water to make a working solution for use.
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 Ammoniac Silver 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.