乙酰胆碱酯酶染色液(亚铁氰化铜法)

货号：G2110
规格：2×20mL
保存：-20℃，避光保存，有效期 6 个月。

产品组成：

名称		2×20mL	保存
试剂(A):AChE孵育液	A1: AChE Iodide	10mg	2-8℃，避光
	A2: AChE Buffer A	2mL	室温
	A3: AChE Buffer B	14ml	2-8℃，避光
	A4: AChE Buffer C	2mL	2-8℃，避光
	A5: AChE Buffer D	2mL	2-8℃，避光
	A6: Iso-OMPA	0.4mL	-20℃，避光
试剂(B):苏木素染色液		20mL	2-8℃，避光
试剂(C): AChE-ChE抑制剂		0.4mL	2-8℃，避光

产品介绍：

胆碱酯酶(cholinesterase，ChE)属于特异性酯酶，可分为两大类。一类是乙酰胆碱酯酶(Acetyl cholinesterase，AChE)又称为真性胆碱酯酶，能水解乙酰胆碱，起到生理调节作用；另一类为胆碱酯酶，又称假性胆碱酯酶(Pseudo cholinesterase，PsChE)，能水解胆碱的酯而不能水解乙酰胆碱酯。乙酰胆碱酯酶主要存在于神经元的胞质内、神经不肌肉接头处即所谓运动终板处；PsChE主要存在于血浆、胰腺、唾液腺内，生理功能尚不明确。

乙酰胆碱酯酶染色液(亚铁氰化铜法)属于Karnovsky和Roots法，其染色原理是乙酰胆碱酯酶水解碘化乙酰硫代胆碱，释放乙酸和硫代胆碱。硫代胆碱中的硫氢基(-SH)把铁氰化钾还原为亚铁氰化钾，后者与铜离子结合形成不溶性的红棕色至深棕色的亚铁氰化铜沉淀在酶活性部位而显示出来。其优点是操作简便、酯酶的扩散较少，其缺点是对底物对组织的渗透性较差。该染色液可用于观察中枢神经和周围神经纤维等疾病情况下的改变，亦有利于巨结肠症、肠神经元发育异常的诊断。有机农药中毒时可使该酶受到抑制，酶的活性下降而呈阴性反应。

自备材料：

10%甲醛钙固定液、恒温培养箱、光学显微镜

操作步骤：(*仅供参考*)

1、 冰冻切片，厚6μm，不固定或置于遇冷的10%甲醛钙固定10min。
2、 蒸馏水洗3次，每次1min。
3、 配制AChE孵育液：临用前，取A2加入至A1中，使后者完全溶解，即为A12混合液，4℃保存。取适量的A12混合液、A3、A4、A5、A6，按A12混合液:A3:A4:A5:A6=1:7:1:1:0.2充分混合，即为AChE孵育液，6h内使用。注意：如果想显示AChE和ChE，即无需区分AChE和ChE，无需加入Iso-OMPA。
4、 切片入预温的AChE孵育液中，37℃避光孵育1-3h(一般不超过6h)，至切片呈淡棕色时取出。
5、 蒸馏水洗，镜下观察如活性着色较淡，可于进行二次孵育，至反应合适为止。流水冲洗5min。
6、 滴加苏木素染色液浅染细胞核3-5min。流水冲洗10min。
7、 常规脱蜡透明，中性树胶封固。

染色结果：

AChE酶活性部位	红棕至深棕色
细胞核	蓝色

阴性对照(*可选*)： 取配制好的AChE孵育液，按AChE孵育液: AChE-ChE抑制剂=50:1充分混合。取相同切片入含AChE抑制剂的AChE孵育液中，其余同上，呈阴性反应。

注意事项：

1、 本染色液适用于冰冻切片，同时应减少切片在室温暴露的时间。

2、 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Acetylcholinesterase Stain Kit(Copper Ferricyanide)

Cat: G2110
Size: 2×20mL
Storage: -20℃, avoid light, valid for 6 months.

Kit Components

Reagent		2×20mL	Storage
Reagent (A): AChE Incubation Solution	A1: AChE Iodide	10mg	4℃, avoid light
	A2: AChE Buffer A	2mL	RT
	A3: AChE Buffer B	14mL	4℃, avoid light
	A4: AChE Buffer C	2mL	4℃, avoid light
	A5: AChE Buffer D	2mL	4℃, avoid light
	A6: Iso-OMPA	0.4mL	-20℃, avoid light
Reagent (B):Hematoxylin Solution		20mL	4℃, avoid light
Reagent (C): AChE-ChE Inhibitor		0.4mL	4℃, avoid light

Introduction

Cholinesterase(ChE) belongs to specific esterase and can be divided into two categories. One is acetylcholinesterase, also known as true cholinesterase, which can hydrolyze acetylcholine and play a physiological regulatory role; the other is cholinesterase, also known as Pseudo cholinesterase(PsChE), which can hydrolyze choline esters instead of acetylcholinesterase. Acetylcholinesterase mainly exists in the cytoplasm of neurons, the junction of nerves and muscles which also called motor endplate; PsChE mainly exists in plasma, pancreas and salivary gland, but its physiological function is not clear.

Acetylcholinesterase Stain Kit(Copper Ferricyanide) belongs to Karnovsky and Roots method. Its dyeing principle is that acetylcholinesterase hydrolyzes acetylcholine iodide and releases acetic acid and thiocholine. The thiohydrogen group (- SH) in thiocholine reduces potassium ferricyanide to potassium ferrocyanide, which combines with copper ions to form insoluble red brown to dark brown copper ferrocyanide precipitated in the active site of the enzyme. Its advantages are simple operation, less esterase diffusion, and its disadvantages are poor permeability of substrate to tissue. The staining solution can be used to observe the changes under central and peripheral nerve fibers diseases, and it is also helpful for the diagnosis of megacolon and intestinal neuron dysplasia. When poisoned by organic pesticides, the enzyme is inhibited and the activity of the enzyme decreased with the negative reaction.

Self Provided Materials

10% Formaldehyde Calcium Fixative, Constant temperature incubator, Optical microscope

Protocol(*for reference only*)

1. Cut frozen section in 6μm thickness, unfix or fix in precooled 10% Formaldehyde Calcium Fixative for 10mins.
2. Wash with distilled water for 3 times and each time for 1 min.
3. Prepare AChE Incubation Solution: before use, take A2 and add into A1 to make the latter solution completely dissolved to form A12 mixture, and store at 4℃. Take appropriate amount of A12 mixture, A3, A4, A5, A6, and mix them fully as the radio of 1:7:1:1:0.2 to form AchE Incubation Solution, which shall be used within 6h.
   Note: if you want to display AChE and ChE, you do not need to distinguish AChE and ChE, and you do not need to add Iso-OMPA.
4. Take the section into the preheated AChE Incubation Solution and incubate at 37℃ in dark for 1-3 h (generally no more than 6 h), then take out when the section is light brown.
5. Wash with distilled water. View under the optical microscope, if the color of active site is still light, can incubate after washing with distilled water until the reaction is appropriate.
6. Rinse with running water for 5mins.
7. Redyeing with Hematoxylin Solution for 3-5mins.
8. Rinse with running water for 10mins.

9. Conventionally dewax in alcohol and transparent by xylene, then seal with resinene.

Result

Active site of AchE enzyme	Red Brown to dark Brown
Nucleus	Blue

Negative control(*optional*)： Take the prepared AChE Incubation Solution and mix it with AChE-ChE Inhibitor as the radio of 50:1. Take the same section and put it into the AChE Incubation Solution containing AChE inhibitor, then follow the steps as the same as above. The result is negative reaction.

Note

1. The staining solution is suitable for frozen sections, and the exposure time of sections at room temperature should be reduced.
2. For your safety and health, please wear experimental clothes and disposable gloves.