乙酰胆碱酯酶染色液(金属沉淀法)

货号：G2111
规格：3×20mL
保存：-20℃，避光保存，有效期 6 个月。

产品组成：

名称		3×20mL	保存
试剂(A): AChE 孵育液	A1: AChE Iodide	48mg	2-8℃，避光
	A2: AChE Buffer A	18.4mL	室温
	A3: AChE Buffer B	0.8mL	2-8℃，避光
	A4: AChE Buffer C	0.8mL	2-8℃，避光
	A5: Iso-OMPA	0.4mL	-20℃，避光
试剂(B): AChE 漂洗液		2×20mL	室温
试剂(C): AChE 硫化液		2×1mL	室温，避光
试剂(D): AChE-ChE 抑制剂		0.4mL	2-8℃，避光

产品介绍：

胆碱酯酶(cholinesterase)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，起到生理的调节作用；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、神经与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。显示乙酰胆碱酯酶的方法有 Koell 法、Snell 和 Garrett 法、Karnovsky 和 Roots 法等。

乙酰胆碱酯酶染色液(金属沉淀法)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，磷酸盐与铅离子形成磷酸铅沉淀，再被 S^{2+}置换，形成硫化铅沉淀。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，有可能出现假阳性。

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 6um，不固定或置于预冷的 10%甲醛钙固定。
2. 蒸馏水洗 2 次，每次 3min。
3. 配制 AChE 孵育液：临用前，取 AChEIodide buffer 加入至 AChEIodide，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，按 A12 混合液：A3：A4:A5=46:2:2:1 充分混合，即为 AChE 孵育液，3h 内使用。
4. 切片入预温的 AChE 孵育液中，37℃避光孵育 10-30min。
5. 蒸馏水洗 5min，镜下观察如活性部位仍较淡，可于蒸馏水洗后再进行孵育，至反应合适为止。
6. 入 AChE 漂洗液充分漂洗，如 AChE 漂洗液用量过大，可用 PBS 代替。
7. 在上述过程中配制 ALP 硫化工作液，即取适量的试剂 C 用蒸馏水或者去离子水稀释 50 倍，即为 ALP 硫化工作液，即配即用。切片入 ALP 硫化工作液孵育。
8. 流水冲洗 10min。甘油明胶封片。

染色结果：

AChE 酶活性部位	棕黑色

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-ChE 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(Lead Copper Sulphide Choline)

Cat: G2111
Size: 3×20mL
Storage:-20℃, avoid light, valid for 6 months.

Kit Components

Reagent		3×20mL	Storage
Reagent(A): AChE Incubation Solution	A1: AChE Iodide	48mg	2-8℃, avoid light
	A2: AChE Buffer A	18.4mL	RT
	A3: AChE Buffer B	0.8mL	2-8℃, avoid light
	A4: AChE Buffer C	0.8mLl	2-8℃, avoid light
	A5: Iso-OMPA	0.4mL	-20℃, avoid light
Reagent (B): AchE Washing Solution		2×20mLl	RT
Reagent (C): AchE Vulcanizing Solution		2×1mL	RT, avoid light
Reagent (D): AChE-ChE Inhibitor		0.4mL	2-8℃, 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. The methods to display acetylcholinesterase areKoell method, Snell method, Garrett method, Karnovsky method and Roots method.

The dyeing principle of this kit is that acetylcholinesterase hydrolyzes acetylthiocholine iodide, releases acetic acid and thiocholine, phosphate and lead ions form lead phosphate precipitation, and then replaced by S^{2+} with lead sulfide precipitation. Its advantages are simple operation, less esterase diffusion, and its disadvantages are poor permeability of substrate to tissue, which may lead to false positive.

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 2 times and each time for 3mins.
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, and mix them fully as the radio of 46:2:2:1 to form AchE Incubation Solution, which shall be used within 3h.
4. Take the section into the preheated AChE Incubation Solution and incubate at 37℃ in dark for 10-30mins.
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. Add the section into AchE Washing Solution and fully wash. If the volume for use is more, can replace with PBS.
7. In the above process, take a proper amount of Reagent C and dilute it 50 times with distilled water or deionized water to prepare AchE Vulcanizing Working Solution. It is ready to use. Incubate the section in AchE Vulcanizing Working Solution.
8. Rinse with running water for 10mins. Seal with glycerin gelatin.

Result

Active site of AchE enzyme	Black Brown

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.