乙酰胆碱酯酶染色试剂盒(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 孵育工作液	试剂(A1): AChE 碘化底物	48mg	2-8℃, 避光
	试剂(A2): AChE 缓冲液A	18.4mL	室温
	试剂(A3): AChE 缓冲液B	0.8mL	2-8℃, 避光
	试剂(A4): AChE 缓冲液C	0.8mL	2-8℃, 避光
	试剂(A5): AChE 敏化剂	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；另一类称为假性胆碱酯酶(Pseudo cholinesterase，PsChE)，能水解其他胆碱脂类如琥珀胆碱。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。

乙酰胆碱酯酶染色试剂盒(金属沉淀法)染色原理是真性胆碱酯酶水解专一性底物释放硫代胆碱，硫代胆碱的硫醇基团可以和重金属离子形成初级络合物沉淀，散在的分布在酶活性位点及其周围，经充分清洗去杂后初级沉淀与硫化液发生复分解，生成更精细准确的二次沉淀定位。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，对操作者经验要求较高，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，推荐厚 6-12um，不固定直接复温晾干或置于预冷的 10%甲醛钙固定液固定 10-15min。
2. 蒸馏水洗 2 次，每次 3min。
3. 配制 AChE 孵育液：临用前，取 500ul 试剂(A2): AChE 缓冲液 A 加入至试剂(A1): AChE 碘化底物中，吹打数次使后者完全溶解后全部吸出转移至 A2 瓶中混匀，即为 A12 混合液，保存见注意事项 2。取适量的 A12 混合液、A3、A4、A5 放置复温后，按 A12 混合液：A3：A4:A5=46:2:2:1 充分混合制备 AChE 孵育液，建议 3h 内使用。
4. 切片入恢复室温的 AChE 孵育工作液中，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，可于蒸馏水洗后再新配孵育液进行孵育，至合适为止。
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。（见注意事项 3）

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 孵育工作液，按 AChE 孵育工作液: 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. A12 混合液可在 2-8℃保存 1 周，如需长期保存建议分装成小规格放置-20℃保存。A5 同理，长期保存建议分装冻存，避免反复冻融对染色的影响。
3. 试剂 C：AChE 硫化液有强烈气味，建议在通风橱进行稀释或染色操作。
4. 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(Metal Precipitation Method)

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 belongs to specific esterases and can be divided into two categories. One is acetylcholinesterase (AChE), also known as true cholinesterase, which can hydrolyze acetylcholine and play a physiological regulatory role; The other is pseudocholinesterase (psche), which can hydrolyze other cholinergic lipids such as succinylcholine. Acetylcholinesterase mainly exists in the cytoplasm of neurons and at the junction of nerve and muscle, which is the so-called motor endplate; Psche mainly exists in plasma, pancreas and salivary gland, and its physiological function is unclear. The methods to display acetylcholinesterase include koell method, Snell and Garrett method, Karnovsky and roots method, etc.

The dyeing principle of Acetylcholinesterase Stain Kit(Metal Precipitation Method) is that true cholinesterase hydrolyzes the specific substrate to release thiocholine. Thiol groups of thiocholine can form primary complex precipitation with heavy metal ions, which are scattered around the active site of the enzyme. After thorough cleaning and removal of impurities, the primary precipitation and sulfide solution undergo double decomposition to generate more precise and accurate secondary precipitation localization. Its advantages are simple operation and less diffusion of esterase, but its disadvantages are poor permeability of substrate to tissue, high requirements for operator experience and possible 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°C 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.