退化神经元检测试剂盒

货号：G3262
规格：3×10mL/3×50mL
保存：2-8℃，避光保存，有效期 6 个月

产品组成：

名称		3×10mL	3×50mL	保存
试剂(A):预处理液 A（10×）		10mL	50mL	室温，避光
临用前取适当体积试剂 A 用 90%乙醇稀释 10 倍后使用，稀释的工作液可冷藏保存 48h，建议现配现用。				
试剂(B):预处理液 B（10×）		10mL	50mL	室温，避光
临用前取适当体积试剂 B 用蒸馏水稀释 10 倍后使用，稀释的工作液可冷藏保存 48h，建议现配现用。				
试剂(C):工作液	试剂(C1):储备液（10×）	1mL	5mL	2-8℃，避光
	试剂(C2):稀释液	9mL	45mL	2-8℃，避光
临用前取出试剂 C1、C2 复温至室温（25~30℃）后按照 1：9 的比例配制成工作液 C，建议在 4h 内使用。				

产品介绍：

神经元变性的原因和影响是神经科学家们的主要关注点，目前已有多种检测神经元变性的方法，包括镀银染色和 FJC 染色方法。FJC 染色是近年来新兴的一种神经元荧光染色技术，不仅避免了常规镀银染色的繁琐和高成本，而且可以与其它荧光素标记物结合进行多重荧光染色。相较于其他染料，Fluoro-Jade C 具有更高的信号背景比，以及更高的分辨率。这使其不仅可以定位神经细胞体的退化，还可以定位远端树突，轴突和末端。而且该染料具有高度抗褪色性，几乎与所有组织学处理和染色方案兼容。

退化神经元检测试剂盒可对退化神经元特异染色，本染色试剂盒提供成套的即用型试剂套装，可在简单稀释后，可直接用于实验，方便快捷。

自备材料：

多聚赖氨酸载玻片、染色缸、盖玻片、中性树胶、恒温箱、系列乙醇、二甲苯

操作步骤：*(仅供参考)*

1. 冰冻切片浸于蒸馏水复温 3min，石蜡切片脱蜡至水。
2. 切片自然晾干。
3. 浸入稀释好的试剂 A 工作液中 5min，然后转入 70%乙醇 2min，然后蒸馏水浸洗 2min。
4. 浸入稀释好的试剂 B 工作液中漂白 10min。
5. 蒸馏水洗 2min。
6. 将配好的试剂 C 工作液均匀滴加在处理后的脑切片上，室温避光孵育 10min。
7. 蒸馏水洗三次，每次 1min。
8. 晾干、透明（二甲苯 1min），中性树胶封片。
9. 在荧光显微镜下观察。（使用蓝光或者 488nm 激光激发，FITC 通道检测）

染色结果：

退化神经元	绿色荧光（FJC）
细胞核	蓝色荧光（DAPI）

注意事项：

1. 该试剂盒可染色切片数量取决于单次染色容器大小。以能装 5 张切片的标准剥离染色缸为例，每 50ml 稀释好的试剂 C 工作液可以染色 80-100 张切片，滴染建议是 400-600ul/张。稀释后的工作液不稳定，单次试验建议在 4h 内使用完毕，每次开始实验批次时，最好使用新稀释的试剂 C 工作液。
2. 试剂盒内试剂建议根据说明书标注保存温度分开保存，以免反复冻融对染色效果造成影响。
3. FJC 为曙红类似物（着色红细胞），可能会对血管有一定染色效果，可以通过组织灌注或其他组织预处理步骤来有效避免血管染色，但可能无法完全消除。从测试结果来看，明显的形态学差异通常不会对结果判读造成影响。
4. 染色后清洗、封片、镜下观察的过程中注意避免强光照射。
5. 试剂原液或稀释液可能有少量沉淀，通常在稀释过程中会溶解，不影响染色结果。如有顾虑可用针头过滤器（水性滤膜）过滤后使用。
6. 通常建议使用正常小鼠脑切片作为阴性对照，应无明显染色。

Degenerating Neurons Staining Kit (FJC Method）

Cat: G3262
Size: 3×10mL/3×50mL
Storage: 2-8℃, avoid light, valid for 6 months.

Kit components

Reagent		3×10mL	3×50mL	Storage
Reagent(A):Pretreatment Solution A（10×）		10mL	50mL	RT, avoid light
Before use, pick appropriate amount of Reagent(A) and dilute 10 times with 90% ethanol to form Pretreatment Solution A（1×）, wihich is can be kept in cold storage for 48h. It is recommended to prepare for timely use.				
Reagent(B):Pretreatment Solution B（10×）		10mL	50mL	RT, avoid light
Before use, pick appropriate amount of Reagent(B) and dilute 10 times with distilled water to form Pretreatment Solution B（1×）, wihich is can be kept in cold storage for 48h. It is recommended to prepare for timely use.				
Reagent(C):Working Solution C	C1:Stock Solution（10×）	1mL	5mL	2-8℃, avoid light
	C2:Diluent	9mL	45mL	2-8℃, avoid light
Before use, take out C1, C2 and restore to room temperature（25~30℃）. Then mi× C1 with C2 as the ratio of 1:9 to formWorking Solution, which is recommended to use within 4hour。				

Introduction

The causes and effects of neuron degeneration are the main concerns of neuroscientists. At present, there are many methods to detect neuron degeneration, including silver plating staining and FJC staining. FJC staining is a new fluorescence staining technique for neurons in recent years. It not only avoids the cumbersome and high cost of conventional silver plating staining, but also can be combined with other fluorescein markers for multiple fluorescence staining. Compared with other dyes, fluoro jade C has higher signal background ratio and higher resolution. This allows it to locate not only the degeneration of nerve cell bodies, but also distal dendrites, a×ons and terminals. Moreover, the dye has high fading resistance and is compatible with almost all histological treatment and dyeing schemes.

Degenerative Neuron Detection Kit (FJC Method) can specifically stain degenerative neurons. This staining kit provides a complete set of ready to use reagent kit, which can be directly used in e×periments after simple dilution, which is convenient and fast.

Self Provided Materials

Polylysine slide, dyeing vat, cover glass, resinene, incubator, series ethanol, ×ylene

Protocols*(for reference only)*

1. For frozen section, immerse in distilled water to rewarm for 3min; For paraffin section, dewa× to water.
2. Let dry naturally.
3. Immerse in the Pretreatment Solution A（1×） for 5min, then transfer into 70% ethanol for 2min, and then immerse in distilled water for 2min.
4. Immerse in Pretreatment Solution B（1×）for bleaching for 10min.
5. Wash with distilled water for 2min.
6. Add prepared Working Solution C evenly onto the treated brain slices and incubate at room temperature in the dark for 10 minutes.
7. Wash three times with distilled water for 1min each time.

8. Dry and transparent (×ylene for 1min) and seal with resinene.
9. View under fluorescence microscope. (e×cited by blue light or 488nm laser and detected by FITC channel)

Result

Degenerative neuron	Green fluorescence（FJC）
Nuclear	Blue fluorescence（DAPI）

Note

1. The number of sections that can be stained by the kit depends on the size of the single staining container. Taking the standard stripping dyeing cylinder capable of holding 5 slices as an e×ample, every 50ml of diluted Working Solution C can dye 80-100 slices, and the drip dyeing suggestion is 400-600ul/ piece. The diluted working solution is unstable. It is recommended to use it within 4h for a single test. It is best to use the newly diluted Working Solution C when starting the test batch each time.
2. It is recommended to store the reagents in the kit separately according to the storage temperature marked in the manual to avoid repeated freezing and thawing affecting the dyeing effect.
3. FJC is a eosin analogue (colored red blood cells), which may have a certain staining effect on blood vessels. It can effectively avoid blood vessel staining through tissue perfusion or other tissue pretreatment steps, but it may not be completely eliminated. From the test results, obvious morphological differences usually do not affect the interpretation of the results.
4. In the process of cleaning, sealing and microscopic observation after dyeing, pay attention to avoid strong light irradiation.
5. There may be a small amount of precipitation in reagent stock solution or diluent, which will usually dissolve in the dilution process and will not affect the dyeing results. If you have concerns, you can use the needle filter (water-based filter membrane) after filtration.
6. It is generally recommended to use normal mouse brain slices as negative control, and there should be no obvious staining.