V02

一氧化氮合酶染色试剂盒

货号：G3400
规格：5×20mL
保存：2-8℃，避光保存，有效期 6 个月。

产品组成：

名称	5×20mL	保存
试剂(A): Tissue PB buffer	100mL	室温
试剂(B): Cell PB buffer	50mL	室温
试剂(C):Wash buffer(6×)	20mL	室温
试剂(D): NOS 孵育液	20mL	-20℃，避光
试剂(E): 中性红染色液	20mL	室温，避光

产品介绍：

细胞中的左旋精氨酸在一氧化氮合酶(Nitxic oxide synthase，NOS)的作用下生成一氧化氮和瓜氨酸。一氧化氮合酶染色液包含磷酸盐缓冲、漂洗、孵育、复染等步骤，可用于组织冰冻切片染色，尤其适用于脑组织冰冻切片染色，亦可用于细胞爬片、细胞涂片染色。

操作步骤：*(仅供参考)*

(一)、脑组织冰冻切片：
1. 动物常规灌注固定，取脑组织，浸入 30%蔗糖溶液，冰冻切片厚度 40μm。
2. 入 Tissue PB buffer 漂洗 10min，重复 1 次。
3. 用蒸馏水稀释 Wash buffer(6×)至 1×，切片入 1×Wash buffer，室温孵育 60min。
4. 入 NOS 孵育液，并放入湿盒中，37℃避光孵育 3h。
5. 用蒸馏水稀释 Wash buffer(6×)至 3×，切片入 3×Wash buffer，4℃孵育过夜。
6. 入 Tissue PB buffer，漂洗 10min，重复 1 次。
7. 裱片、晾干。
8. 可选步骤：入中性红染色液复染 1～2min。
9. 常规脱水、透明、封片、镜检。

(二)、细胞爬片：
1. 细胞爬片或甩片用 Cell PB buffer 漂洗 5min，重复 1 次。
2. 4%多聚甲醛室温固定 30min。
3. 入 Cell PB buffer 漂洗 10min，重复 1 次。
4. 按上述脑组织切片步骤 3-5 操作。
5. 入 Cell PB buffer 漂洗漂洗 10min，重复 1 次。
6. 可选步骤：入中性红染色液复染 1～2min。
7. 封片、镜检。

染色结果：

NOS 部位	蓝黑色
背景	红色(中性红)或淡蓝色

注意事项：

1. 应选择恰当的固定液、固定方法、固定时间，否则会影响酶的活性。
2. 中性红复染可以更好的显示细胞轮廓，有助于进一步计数阳性细胞率。
3. 组织切片染色时，可见 NOS 神经元，类似于 Golgi 银染，胞体、神经纤维、纤维末梢均可着色。

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Nitric Oxide Synthetase/NOS Stain Kit

Cat: G3400
Size: 5×20mL
Storage:2-8℃, avoid light, valid for 6 months.

Kit Components

Reagent	5×20mL	Storage
Reagent(A): Tissue PB Buffer	100mL	RT
Reagent(B): Cell PB Buffer	50mL	RT
Reagent(C):Wash Buffer(6×)	20mL	RT
Reagent(D): NOS Stain Solution	20mL	-20℃, avoid light
Reagent(E): Neutral Red Staining Solution	20mL	RT, avoid light

Introduction

L-arginine in cells generates nitric oxide and citrulline under the action of nitric oxide synthase (NOS). The nitric oxide synthase staining solution contains steps such as PB treating, rinsing, incubation, and counterstaining, and can be used for staining frozen tissue sections, especially for staining frozen brain tissue sections. It can also be used for staining cell slides and cell smears.

Protocol(*for reference only*)

Frozen section of brain tissue:

1. Animal routine perfusion fixation, brain tissue taken, immersed in 30% sucrose solution, frozen section thickness of 40μ m.
2. Rinse in Tissue PB Buffer for 10 minutes and repeat once.
3. Dilute the Wash Buffer (6×) with distilled water to 1×, slice into 1×Wash Buffer, and incubate at room temperature for 60 minutes.
4. Soak the section in NOS Stain Solution and incubate at 37 ℃ in the dark for 3 hours.
5. Dilute the Wash Buffer (6×) with distilled water to 3×, slice into 3×Wash Buffer, and incubate overnight at 4 ℃.
6. Transfer to Tissue PB buffer, rinse for 10 minutes, repeat once.
7. Film mounting and air drying.
8. Optional steps: Repeat staining with Neutral Red Staining Solution for 1-2 minutes.
9. Conventional dehydration, transparency, film sealing, and microscopic examination.

Cell crawling slides:

1. Rinse the cell slide or shake slide with Cell PB Buffer for 5 minutes, repeat once.
2. Fix with 4% paraformaldehyde at room temperature for 30 minutes.
3. Rinse in Cell PB buffer for 10 minutes and repeat once.
4. Follow steps 3-5 of the above brain tissue sectioning procedure.
5. Rinse in Cell PB buffer for 10 minutes, repeat once.
6. Optional steps: Repeat staining with Neutral Red Staining Solution for 1-2 minutes.

Result

NOS site	Blue Black
Background	Red (Neutral Red) or Light Blue

Note

1. Appropriate fixative, fixation method, and fixation time should be selected, otherwise it will affect the activity of the enzyme.
2. Neutral red counterstaining can better display cell contours and help further count positive cell rates.
3. When staining tissue sections, NOS neurons can be seen, similar to Golgi silver staining, with staining of the cell body, nerve fibers, and fiber endings.