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细胞色素氧化酶染色试剂盒(对苯二铵法)

货号: G2411 规格: 4×50mL

保存: 2-8℃, 避光保存, 有效期 6 个月。

试剂组成:

名称		4×50mL	保存
试剂(A): CO 孵育液		50mL	2-8℃,避光
试剂(B): Lugol 碘液		50mL	室温,避光
试剂(C): 海波溶液		50mL	室温
试剂(D):	D1: CO 分化液 A	25mL	室温,避光
CO 分化液	D2: CO 分化液 B	25mL	室温
临用前,取 D1、D2 等量混合,即为 CO 分化液,即配即用。			
试剂(E): CO 对照液		10mL	2-8℃,避光

产品介绍:

细胞色素氧化酶(Cytochrome Oxidase, CO)被认为是线粒体膜固有的酶,在含有大量线粒体的细胞(如心肌、肾小管上皮以及胃壁细胞、肝细胞)内都具有高度活性。

细胞色素氧化酶染色试剂盒(对苯二铵法)以 N-苯基-对-苯二胺为底物,在有氧存在的情况下,经细胞色素氧化酶作用可与萘酚生成有色的靛酚蓝,即为 Nadi 反应。

操作步骤: (仅供参考)

- 1. 冰冻切片,厚6µm,不固定。
- 2. 切片入 CO 孵育液内,室温(20-25℃)孵育 20-60min。
- 3. 切片入 Lugol 碘液, 孵育 2-3min。
- 4. 入海波溶液处理 1-5min。
- 5. 入配制好的 CO 分化液, 分化 30s。 流水冲洗 3min。
- 6. 常规脱水透明,中性树胶封固。

染色结果:

CO酶活性部位	蓝褐色
心肌、肾小管上皮内颗粒(线粒体)	棕色

阴性对照(可选):

相同切片入试剂(E) CO 对照液中,室温孵育 20~60min,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. CO 孵育液孵育时间因组织而异,心肌、肾孵育约 20-30min, 肝脏约 50-60min, 甲状腺滤泡上皮约 2h。
- 3. 为了您的安全和健康,请穿实验服并戴一次性手套操作。

Cytochrome Oxidase Stain Kit (Nadi Method)

Cat: G2411 Size: 4×50mL

Storage: 2-8°C, avoid light, valid for 6 months.

Kit Components

Reagent		4×50mL	Storage
Reagent(A): CO Incubation Solution		50mL	2-8°C, avoid light
Reagent(B): Lugol Iodine Solution		50mL	RT, avoid light
Reagent(C): Hypo Solution		50mL	RT
Reagent(D):CO	D1:Differentiation Solution A	25mL	RT, avoid light
Differentiation Solution	D2:Differentiation Solution B	25mL	RT
Before use, mix D1 with D	2 in equal amount to form CO Diff	ferentiation S	Solution. It is ready to use.
Reagent(E): CO Control Solution		10mL	2-8°C, avoid light

Introduction

Cytochrome oxidase (CO) is considered to be an intrinsic enzyme of mitochondrial membrane, which has high activity in cells containing a large number of mitochondria (such as cardiac muscle, renal tubular epithelium, gastric parietal cells and liver cells).

The principle of this kit is that take N-phenyl-P-phenylenediamine as the substrate, in the presence of oxygen, it can generate colored indophenol blue with naphthol through the action of cytochrome oxidase, which is called Nadi reaction.

Protocol(for reference only)

- 1. Cut frozen section in 6 μm thickness and unfix.
- 2. Put the section in CO Incubation Solution and incubate it at room temperature (20-25 °C) for 20-60mins.
- 3. Incubate in Lugol Iodine Solution for 2-3mins.
- 4. Treat the section with Hypo Solution for 1-5mins.
- 5. Differentiate by prepared Co Differentiation Solution for 30s.
- 6. Rinse with running water for 3mins.
- 7. Dehydrate in ethanol and transparent by xylene, finally seal with resinene.

Result

Active site of CO enzyme	Bluish Brown
Myocardium, intraepithelial granules of renal tubules(mitochondria)	Brown

Negative control (optional):

Take the same section into Reagent(E)-Co Control Solution and incubate at room temperature for 20-60mins, the rest follow the above steps. It shows negative reaction.

Note

- The staining solution is suitable for frozen section and the exposure time of sections at room temperature should be reduced.
- 2. The incubation time of CO Incubation Solution varies with tissues. The hearts and kidneys are incubated for about 20-30 mins, the liver for about 50-60 mins, and the thyroid follicles for about 2 h.
- 3. For your safety and health, please wear experimental clothes and disposable gloves.