V02

# 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): CO 分化液 | D1: CO 分化液 A | 25mL | 室温，避光 |
| | 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. 切片滴加 CO 孵育液，室温(20-25℃)孵育 20-60min。
3. 切片滴加 Lugol 碘液，孵育 2-3min。
4. 切片滴加海波溶液处理 1-5min。
5. 切片滴加配制好的 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。



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# Cytochrome Oxidase Stain Kit (Nadi Method)

**Cat:** G2411
**Size:** 4×50mL
**Storage:** 2-8℃, avoid light, valid for 6 months.

## Kit Components

| Reagent | | 4×50mL | Storage |
|---|---|---|---|
| Reagent(A): CO Incubation Solution | | 50mL | 2-8℃, avoid light |
| Reagent(B): Lugol Iodine Solution | | 50mL | RT, avoid light |
| Reagent(C): Hypo Solution | | 50mL | RT |
| Reagent(D):CO Differentiation Solution | D1:Differentiation Solution A | 25mL | RT , avoid light |
| | D2:Differentiation Solution B | 25mL | RT |
| Before use, mix D1 with D2 in equal amount to form CO Differentiation Solution. It is ready to use. | | | |
| Reagent(E): CO Control Solution | | 10mL | 2-8℃, 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. Sections were incubated dropwise with incubation solution CO and incubate it at room temperature (20-25 ℃) for 20-60mins.
3. Slices with drops of Lugol's iodine solution for 2-3mins.
4. The slices were treated dropwise with seaborne solution for 1-5 min.
5. The slices were dropwise added to the prepared CO differentiation solution and differentiated for 30s.
6. 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

1. 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.



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