细胞色素氧化酶染色液(联苯胺法)

货号：G2410
规格：4×50mL
保存：-20℃，避光保存，有效期6个月。

产品组成：

名称		规格	保存
试剂(A): CO清除液		2×50mL	2-8℃，避光
试剂(B): DAB孵育液	B1: DAB 染色液	45mL	-20℃，避光
	B2: DAB 增强剂	5mL	-20℃，避光
	B3: DAB 反应液	100μL	-20℃，避光
按B1:B2:B3=9000:1000:2混合，即为DAB孵育液，即配即用。			
试剂(C):苏木素染色液		50mL	2-8℃，避光
试剂(D): CO对照液		1mL	室温

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。此酶活性往往作为细胞内氧化代谢的指标，亦作为线粒体的标志酶之一。

细胞色素氧化酶染色液(联苯胺法)染色原理是细胞色素氧化酶催化 DAB 使其侧链的氨基氧化，进行反复的氧化性聚合和氧化性环化形成不溶性的棕色 Phenazine 聚合物。此酶对固定剂敏感，故须用新鲜切片。

自备材料：

恒温培养箱、光学显微镜

操作步骤：(*仅供参考*)

1、 冰冻切片，厚6μm，不固定。
2、 滴加CO清除剂于切片上，覆盖整个样品表面缓冲2min后倾去。
3、 切片入DAB孵育液中，37℃避光孵育45-60min。
4、 倾去切片上多余染色液，滴加CO清除剂于切片上，覆盖整个样品表面缓冲1min后倾去。
5、 蒸馏水稍洗3-5s。
6、 (*可选*)滴加苏木素染色液浅染细胞核3-5min。
7、 流水冲洗10min。常规脱水透明，中性树胶封固。

染色结果：

CO酶活性部位	棕色
心肌、肾小管上皮内颗粒(线粒体)	蓝色

阴性对照(*可选*)： 取新鲜配制好的DAB孵育液，按DAB孵育液:CO对照液=50:1的比例混合，即为CO对照工作液。相邻切片入CO对照工作液，室温孵育30-60min，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 (DAB Method)

Cat: G2410
Size: 4×50mL
Storage:-20℃, valid for 6 months.

Kit Components

Reagent		Size	Storage
Reagent(A): CO Recover Solution		2×50mL	2-8℃, avoid light
Reagent(B): DAB Incubation Solution	B1: DAB Solution	45mL	-20℃, avoid light
	B2: DAB Reinforcing Solution	5mL	-20℃, avoid light
	B3: DAB Reaction Solution	100μL	-20℃, avoid light
Mix B1, B2 with B3 as the radio of 9000:1000:2 to form DAB Incubation Solution. It is ready to use.			
Reagent(C):Hematoxylin Staining Solution		50mL	2-8℃, avoid light
Reagent(D): CO Control Solution		1mL	RT

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). This enzyme activity is often used as an indicator of intracellular oxidative metabolism, and also as a marker enzyme of mitochondria.

The principle of this kit is that cytochrome oxidase catalyzes the amino oxidation of DAB's side chain, conducts repeated oxidative polymerization and oxidative cyclization to form insoluble brown Phenazine polymer. This enzyme is sensitive to fixative, so must use fresh sections.

Self Provided Materials

Constant temperature incubator, Optical microscope

Protocol(*for reference only*)

1. Cut frozen section in 6μm thickness and unfix.
2. Drop CO Recover Solution onto the section and cover the whole sample surface.
3. Put the section into DAB Incubation Solution and incubate it at 37 ℃ in dark for 45-60mins.
4. Discard the staining solution from the section, drop the CO Recover Solution onto the section, and cover the whole sample surface.
5. Wash slightly with distilled water for 3-5s.
6. (*optional*)Re-dyeing the nucleus with Hematoxylin Staining Solution slightly for 3-5mins.
7. Rinse with running water for 10mins. Dehydrate in ethanol and transparent by xylene, finally seal with resinene.

Result

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

Negative control (*optional*): take fresh prepared DAB Incubation Solution, mix it with CO Control Solution as the ratio of 50:1 to form CO Control Working Solution. Put the adjacent section into CO Control Working Solution and incubate it at room temperature for 30-60mins, and 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 DAB Incubation Solution varies with tissues. The hearts and kidneys are incubated for about 20-30mins, the liver for about 50-60mins, and the thyroid follicles for about 2 h.
3. For your safety and health, please wear experimental clothes and disposable gloves.