

琥珀酸脱氢酶染色试剂盒(四唑盐法)

货号: G2000

规格: 60mL

保存: 2-8°C, 避光保存, 有效期 6 个月。

产品组成:

| 名称 | 60mL | 保存 |
|----------------|------|-----------|
| 试剂(A): NBT 孵育液 | 50mL | 2-8°C, 避光 |
| 试剂(B): NBT 对照液 | 10mL | 2-8°C, 避光 |

产品介绍:

琥珀酸脱氢酶 (succinate dehydrogenase, SDH) 是此琥珀酸氧化酶系的第一个酶, 位于线粒体内。琥珀酸脱氢酶是黄素蛋白酶, 分子内含有-SH, 决定着酶的活性, 故封闭-SH 者皆可作为抑制剂。此酶活性最适 pH 为 7.6~8.5。此酶参与三羧酸循环, 在组织化学上, 常以此酶活性作为三羧酸循环的代表, 亦作为线粒体的标志酶之一。含此酶活性高的组织为心肌、肾小管上皮和肝细胞。此酶对固定剂敏感, 故需用新鲜组织切片。

我司琥珀酸脱氢酶染色试剂盒(四唑盐法)以琥珀酸为底物, 在 SDH 酶作用下脱氢, 硝基蓝四唑(NBT) 为受氢体, 接受氢后被还原为甲噻, 呈蓝紫色, 用以代表琥珀酸脱氢酶的活性。NBT 孵育液含有特殊的中间递氢体, 可使定位更加准确, 染色更加清晰。

自备材料:

蒸馏水、恒温箱或水浴锅

操作步骤: (仅供参考)

1. 取新鲜组织, 不能固定, 制备速冻切片, 切片厚度建议 6 μ m。(见注意事项 1)
2. 向切片上滴加 NBT 孵育液, 置于 37°C 温箱恒温染色 5-30min。蒸馏水轻轻冲洗去除多余染液。
3. 甘油明胶封固。

染色结果:

| | |
|-------|-------|
| 酶活性部位 | 蓝紫色沉淀 |
| 线粒体 | 蓝紫色颗粒 |

阴性对照(可选):

1. 相同切片滴加 NBT 对照液, 置于 37°C 温箱, 孵育约 5-30min, 作为对照。其余步骤同正常步骤, 结果为阴性。
2. (可选) 相同切片经 10% 福尔马林浸泡 30~60min, 再入 NBT 孵育液, 结果为阴性。

注意事项:

1. 本染色液适用于新鲜取材冰冻切片, 组织不能固定, 固定会破坏 SDH 酶活性导致无阳性着色。
2. 对冰冻切片染色时, 应减少切片在室温暴露的时间。
3. 为了您的安全和健康, 请穿实验服并戴一次性手套操作。





Succinate Dehydrogenase Stain Kit(Tetrazole Salt Method)

Cat: G2000

Size: 60mL

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

Kit Components

| Reagent | 60mL | Storage |
|-------------------------------------|------|--------------------|
| Reagent(A): NBT Incubation Solution | 50mL | 2-8°C, avoid light |
| Reagent(B): NBT Control Solution | 10mL | 2-8°C, avoid light |

Introduction

Succinate dehydrogenase (SDH) is the first enzyme of succinate oxidase system, which is located in mitochondria. Succinate dehydrogenase is a flavin protease, containing -SH in the molecule, which determines the activity of the enzyme, so can inhibit it by blocking -SH. The optimum pH of this enzyme was 7.6-8.5. This enzyme is involved in the tricarboxylic acid cycle. In histochemistry, it is often used as the representative of the tricarboxylic acid cycle and one of the marker enzymes of mitochondria. The tissues with high activity of this enzyme are cardiac muscle, renal tubular epithelium and liver cells. This enzyme is sensitive to fixative, so fresh tissue sections are needed.

Succinate Dehydrogenase Stain Kit(Tetrazole Salt Method) uses succinic acid as substrate, dehydrogenates under the action of enzyme, nitro blue tetrazole (NBT) as hydrogen acceptor, after receiving hydrogen, it is reduced to methoxate, which is blue purple, to represent the activity of succinate dehydrogenase. NBT Incubation Solution contains special intermediate transmitters, which can make the location more accurate and the staining more clear.

Self Provided Materials

Distilled water, Incubator or Water bath

Protocol(for reference only)

1. Take fresh tissue, which cannot be fixed, and prepare quick-frozen sections, the thickness of the sections is recommended to be 6 μ m. (See Note 1)
2. Add NBT Incubation Solution onto the section, place in 37°C incubator and stain for about 5-30min.
3. Rinse with distilled water.
4. Glycerin gelatin sealing.

Result

| | |
|-------------------------|-------------------------|
| Positive Site of Enzyme | Blue Purple Precipitate |
| mitochondrion | Blue Purple Particles |

Negative Control (Optional)

1. Place the same sections in NBT control solution directly in 37 °C incubator for about 5-30min. The other steps are the same as the normal steps, and the result is negative.
2. (optional)Soak the same section in 10% formalin for 30-60min and then put into NBT incubation solution, the result is also negative.

Note

1. This kit is suitable for fresh frozen sections, the tissue cannot be fixed, and fixation will destroy the SDH enzyme activity and lead to no positive staining.
2. The exposure time of frozen sections at room temperature should be reduced.
3. For your safety and health, please wear experimental clothes and disposable gloves.

