

植物活性氧检测试剂盒（NBT 法）

货号：G4816

规格：3×100mL

保存：2-8°C，避光保存，有效期 6 个月。

产品组成：

试剂名称	3×100mL	保存
试剂(A):NBT 染色液	100mL	2-8°C，避光
试剂(B):组织脱色液	100mL	室温
试剂(C):组织保存液	100mL	室温

产品介绍：

植物组织在胁迫环境条件下会产生多种活性氧（ROS），ROS 活性非常大且极其不稳定，因此 ROS 的检测通常因其最终产物而定。超氧阴离子是植物活性氧主要组成之一，属于一种含氧自由基，能将 NBT（氮蓝四唑）还原成不溶于水的蓝色甲磺化合物，从而定位组织中的超氧阴离子。

索莱宝植物活性氧检测试剂盒（NBT 法）用于植物活组织中的超氧阴离子染色，一般应用于较嫩的根尖、叶片等的整体染色，染色后有超氧阴离子聚集的部位呈蓝色至深蓝色。

操作步骤：（仅供参考）

- 组织准备：**采集经处理（例如重金属胁迫）的植物幼苗或叶片，自来水洗净，置滤纸上吸干多余水分。
- 组织染色：**将实验样本浸入 NBT 染色液中，-0.1MPa 负压处理 30min，然后室温避光浸染 4-12 h，至阳性部位出现深蓝色，其余部位为淡蓝色或近无色或呈植物本身的颜色即可。（可根据植物幼嫩程度和显色程度调整负压和染色时间）
- 组织脱色：**用镊子将实验小心取出，浸入蒸馏水中来回漂洗 3-5 次，置于滤纸上吸干多余水分后，浸入组织脱色液中于水浴锅 70-80°C 处理 20-40min 直至完全脱去组织背景颜色，处理期间如脱色液颜色较深可更换新鲜的组织脱色液。
- 结果观察：**放凉后取出实验样本，浸入蒸馏水中来回漂洗 3-5 次，置于滤纸上吸干多余水分后，将样本转入适量组织保存液中浸泡 10-30 min，随后可取出拍照。样本可置于保存液中可常温保存月余。

染色结果：

ROS 阳性部位	蓝色或蓝紫色
组织背景（脱色后）	基本无色或淡黄色

注意事项：

- 超氧阴离子容易分解，因此植物样本需要新鲜采集，并尽快完成染色。
- 任何外在刺激因素都可能刺激植物应激产生超氧阴离子，应尽量完整取材避免人为损伤造成假阳性。
- 通常建议脱色时脱色液体积是样本体积的 10 倍以上，可循环使用但颜色太深时建议弃用。
- 在组织样本染色完成后需尽快拍照保存结果。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Plant Tissue ROS Detection Kit(NBT Method)

Cat: G4816

Size: 3×100mL

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

Kit Components

Reagent	3×100mL	Storage
Reagent A:NBT Stain Solution	100mL	2-8°C, avoid light
Reagent B:Decolorization Solution	100mL	RT
Reagent C:Preservation Solution	100mL	RT

Introduction

Plant tissues produce various reactive oxygen species (ROS) under stress environmental conditions, and ROS activity is very high and extremely unstable. Therefore, the detection of ROS is usually determined by its final product. Superoxide anions are one of the main components of plant reactive oxygen species, belonging to an oxygen-containing free radical that can reduce NBT (Nitrotetrazolium Blue) to insoluble blue metal compounds, thereby locating superoxide anions in tissues.

The Solarbio Plant Tissue ROS Detection Kit(NBT Method) is used for superoxide anion staining in plant living tissues. It is generally used for overall staining of tender root tips, leaves, etc. After staining, the areas where superoxide anions accumulate are blue to dark blue.

Protocol(for reference only)

- Organizational Preparation:**Collect plant seedlings or leaves that have been treated (such as heavy metal stress), wash them with tap water, and place them on filter paper to absorb excess water.
- Tissue Staining:**Immerse the experimental sample in NBT staining solution, treat it under negative pressure of -0.1MPa for 30 min, and then immerse it in room temperature and dark light for 4-12 hours until the positive area appears dark blue, and the remaining areas appear light blue or nearly colorless or in the color of the plant itself. (Negative pressure and staining time can be adjusted based on the degree of plant tenderness and color development)
- Tissue Decolorization:**Carefully remove the experiment with tweezers, immerse it in distilled water and rinse back and forth 3-5 times. Place it on a filter paper to absorb excess water, immerse it in the tissue decolorization solution, and treat it in a water bath at 70-80 °C for 20-40 min until the background color of the tissue is completely removed. If the color of the decolorization solution is darker during the treatment, replace it with fresh tissue decolorization solution.
- Result Observation:**After cooling, take out the experimental sample, immerse it in distilled water and rinse it back and forth 3-5 times. Place it on a filter paper to absorb excess water, then transfer the sample to an appropriate amount of tissue preservation solution and soak it for 10 to 30 min. Then, take out the sample and take a photo. The sample can be placed in a storage solution and stored at room temperature for more than a month.

Result

ROS Positive Areas	Blue to Purple
Background(after decolorization)	colorless or light yellow

Note

- Superoxide anions are prone to decomposition, so plant samples need to be freshly collected and stained as soon as possible.
- Any external stimuli may stimulate plant stress to produce superoxide anions, and it is important to obtain complete materials as much as possible to avoid artificial damage and false positives.
- It is usually recommended that the volume of the decolorization solution be more than 10 times the sample volume during decolorization. It can be reused but it is recommended to discard it when the color is too dark.
- After the staining of the tissue sample is completed, it is necessary to take photos and save the results as soon as possible.
- For your safety and health, please wear laboratory clothes and disposable gloves for operation.