

NBT Staining Solution (1mg/mL, pH7.8)

Cat. No.: N1508545 | Pack size: 100 mL | Storage: 2-8°C, protected from light

Overview

Plant tissues produce various reactive oxygen species (ROS) under stress conditions. ROS are highly active and extremely unstable, so their detection is usually based on their end products. Superoxide anion is a type of ROS and belongs to a kind of oxygen-containing free radical. It can reduce NBT (nitroblue tetrazolium) into an insoluble blue formazan compound, thereby localizing superoxide anions in tissues.

NBT staining solution (1 mg/mL, pH 7.8) is composed of NBT and phosphate buffer, and is used for staining superoxide anions in living plant tissues. It is generally applied to whole staining of tender root tips, leaves, etc. After staining, areas with superoxide anion accumulation appear blue to dark blue. This product is for research use only and is not intended for clinical diagnosis or other purposes.

Component

N1508545	Component	100 mL	Storage
N1508545A	NBT	100 mg	2-8°C. Store in the dark.
N1508545B	Phosphate buffer (pH 7.8)	100 mL	2-8°C

Specifications

Property	Information
Synonyms	NBT Stain
Specifications & Purity	BioReagent, Biological Stain, for microscopy, 1 mg/mL
Grade	Biological Stain, BioReagent, for Microscopy
Application	Cell Staining
Sensitivity	Light-sensitive

Storage and Shipping

Property	Information
Storage	Store at 2-8°C, Protected from light
Shipped In	Wet ice
Stability And Storage	Store at 2-8°C long term (12 months). Store in the dark.

Reagents, consumables and Equipments not provided

- Fresh plant leaves or roots, tap water, distilled water, 95% ethanol
- Ultrasonic cleaner, magnetic stirrer, electronic balance, measuring cylinder, filter paper, camera
- NBT staining sample storage solution (optional)

Procedure (For Reference Only)

1. Reagent preparation: Add 100 mg of NBT into 100 mL of phosphate buffer (pH 7.8) and dissolve thoroughly to obtain the NBT staining working solution. Store at 4°C protected from light. It is valid for one month.

Note: If dissolution is difficult, methods such as ultrasonication or magnetic stirring can be used to promote dissolution.

2. Sample preparation: Collect plant seedlings or root tips that have been subjected to stress (e.g., heavy metals). Rinse briefly with tap water and place on filter paper to blot excess moisture.

3. Staining: Immerse the plant seedlings or root tips into the NBT staining working solution. Incubate at room temperature protected from light for 2–6 hours, until positive areas appear dark blue, and the remaining areas are light blue, nearly colorless, or display the plant's original color. (Adjust staining time based on the tenderness and degree of coloration of the plant material.)

4. Decolorization: Carefully remove the plant seedlings or leaves with forceps, immerse them in distilled water and rinse by gently moving back and forth 3–5 times. Place on filter paper to blot excess moisture, then immerse in 95% ethanol and treat at 40°C for 3–16 hours. The purpose is to remove chlorophyll from the plant seedlings or leaves or to reduce the light blue background. During this treatment, replace with fresh 95% ethanol several times.

5. Observation: Remove the plant seedlings or leaves with forceps, immerse them in distilled water and rinse by gently moving back and forth 3–5 times. Place on filter paper to blot excess moisture,

then photograph. (After blotting moisture, samples can be transferred into the NBT staining sample storage solution and soaked for 30 minutes. Samples can be stored in this solution at room temperature for up to one week.)

Notes

1. The prepared NBT staining working solution should be stored at 4°C protected from light and used within one month. Prolonged storage may affect coloration.
2. Since any external factors may stimulate plant stress and generate superoxide anions, plant samples should be freshly collected and stained as soon as possible. It is recommended to include negative and positive blank control groups.
3. Photograph and preserve the results promptly after sample staining is completed.
4. Staining and decolorization steps can also be performed with the following suggested modifications: Place the tissue in the staining solution, apply a vacuum, maintain at -0.1 MPa for 20–30 minutes, then allow to stain statically at room temperature for 60 minutes. Discard the staining solution. Add 95% ethanol and decolorize in a water bath at 70–80°C, replacing the 95% ethanol every 10 minutes. Stop decolorization when the green color of the sample has completely faded.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.

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Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
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