NBT stain

Detect superoxide. Form insoluble dark-blue formazan precipitate after reduced by $O_2^{\bullet -}$.

Invitrogen (N6495), Santa Cruz (sc-296003A)

> Nitro blue Tetrazolium Chloride

NBT working solution (1 mg / mL)

Dissolve the NBT powder in sodium phosphate buffer. The working solution must be **protected from light**.

Chemical	M.W. (g / mol)	Concentration	Addition (mg)	Location
NaH_2PO_4	119.98	1.342 mM	161	III
Na_2HPO_4	141.96	8.657 mM	1228	III
NaN_3	65.01	10 mM	650.1	IV
NBT powder	817.65	—	1000	VII
ddH_2O			1 L	

- The working solution should be filtered before use. The quality among batches is not consistent. Some batches can be fully dissolved in the buffer, resulting clear yellowish working solution (the staining result is dark blue). However, some batches will form reddish brown precipitates (the staining result will be reddish-darkbrown or greyish-darkblue, and tend to be over-stained).

Procedure

1. Directly immersed the root into the NBT solution (pre-warmed to 30°C), avoid the roots from touching the tube wall.
2. Vacumm 25 minutes under ~ 650 mmHg (the lid should be opened).
3. (optional) Close the lid and incubate under 30°C for 10 minutes ~ 2 hours, depend on the root condition.

- Generally, 10 mins is appropritate for young root (less than one week old), and the extremely old root (more than two weeks old) may require 2 hours.

4. Take picture under a dissection or light microscope.

Done !!!

Example image

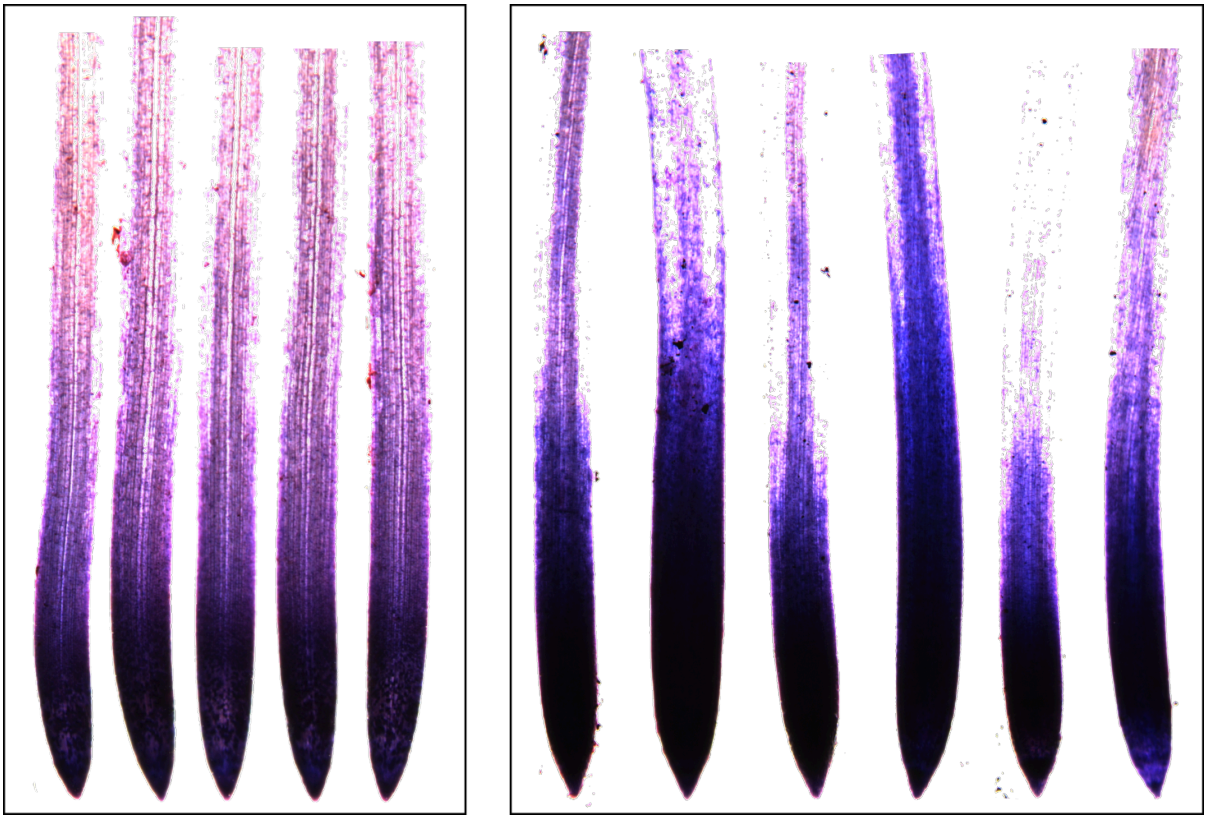


Figure 1. NBT staining in rice roots under different incubation conditions. Non-specific reddish staining occurs when using NBT solutions with excessive precipitate or when incubating at elevated temperatures (e.g., 37°C) (left). The optimized protocol yields characteristic, specific NBT staining (right).