

NBT stain

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

Nitro blue Tetrazolium Chloride

10 mM **sodium phosphate buffer (pH 7.8)**
10 mM **NaN₃ (sodium azide)**
1 mg / mL **NBT powder**

NBT working solution

The working solution must be **protected from light**.

Chemical	M.W. (g / mol)	Concentration	Addition (mg)	Location
NaH ₂ PO ₄	119.98	1.342 mM	161.0	III
Na ₂ HPO ₄	141.96	8.657 mM	1228.0	III
NaN ₃	65.01	10 mM	650.1	IV
NBT powder	817.65	1 mg / mL	1000.0	VII
ddH ₂ O			1 L	

Sodium Phosphate Buffer Preparation and Recipe

Procedure

1. Directly immersed the root into the NBT solution (pre-warmed to 37°C), avoid the roots from touching the tube wall.
 2. Vacuum 20 minutes under ~ 650 mmHg (the lid should be opened).
 3. (optional) Close the lid and incubate under 37°C for 10 minutes ~ 2 hours, depends on the root condition.
- Generally, 10 mins is appropriate for young root (less than one week old), and the extremely old root (more than two weeks old) may require 2 hours.

Note

[Invitrogen \(N6495 \)](#), the working solution should be better 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 dark brown, and seems like tend to be over-stained).

[Santa Cruz \(sc-296003A \)](#), relatively stable.