

Nitroblue tetrazolium (NBT) staining for *Arabidopsis thaliana*

- (1) Cut the leaves of *Arabidopsis* grown on soil or MS plate.
- (2) Put the leaves and NaN_3 (*1) solution into a syringe. Evacuate the air from the syringe.
- (3) Insert the needle of the syringe into a rubber plug.
- (4) Infiltrate the NaN_3 solution completely into the leaves by repeatedly depressing the plunger of the syringe.
- (5) Incubate for 1 minute.
- (6) Remove the NaN_3 solution.
- (7) Add the NBT solution (*2) to the syringe, and infiltrate the NBT solution into the leaves in a similar fashion.
- (8) Incubate for 2 hours at room temperature under light (Or incubate until control plant becomes stained.)
- (9) Decolorize the leaves with AGE solution (*3).

*1: NaN_3 solution

10mM NaN_3

10mM potassium phosphate buffer (pH7.8)

*2: NBT solution

0.1% (v/v) nitroblue tetrazolium (Sigma)

10mM potassium phosphate buffer (pH7.8)

*3: AGE solution

Acetic acid : glycerol : ethanol (1 : 1 : 3 [v/v/v])

Supplementation

- (1) After decolorization you should observe the stained leaves using a microscope as soon as possible.

Reference

Kawai-Yamada M, Ohori Y, Uchimiya H (2004) Dissection of Arabidopsis Bax inhibitor-1 suppressing Bax-, hydrogen peroxide-, and salicylic acid-induced cell death. *Plant Cell* 16:21-32.