Cellular Extract Preparation for Superoxide Dismutase (SOD) Activity Assay

Wen-Yu Kuo, Chien-Hsun Huang, Chun Shih and Tsung-Luo Jinn*

Institute of Plant Biology, National Taiwan University, Taipei, Taiwan
*For correspondence: jinnt@ntu.edu.tw

[Abstract] Superoxide dismutase (SOD) acts as a primary defence against reactive oxygen species (ROS) by converting O^{2-} to O_2 and H_2O_2. Members of this enzyme family include CuZnSOD, MnSOD and FeSOD. Most eukaryotes harbor CuZnSOD and MnSOD, and FeSOD is found in plants and prokaryotes. This protocol is to demonstrate how to prepare the cellular extract for the identification and characterization of SODs *in planta*.

Materials and Reagents

1. Nitroblue tetrazolium (NBT) (Sigma-Aldrich, catalog number: N6876)
2. Riboflavin (Sigma-Aldrich, catalog number: R4500)
3. N,N,N',N'-Tetramethylethylenediamine (TEMED) (Sigma-Aldrich, catalog number: T9281)
4. KCN (Sigma-Aldrich, catalog number: 60178)
5. H_2O_2 (Sigma-Aldrich, catalog number: 349887)
6. NBT solution (see Recipes)
7. Grinding buffer (see Recipes)
8. Riboflavin solution (see Recipes)
9. KCN solution (see Recipes)
10. H_2O_2 solution (see Recipes)

Equipment

1. A light box (white light)
2. Centrifuge (Heraecus, Biofuge fresco)
3. Protein gel cassette

Procedure

A. *Arabidopsis* cellular extract preparation

1. *Arabidopsis* seedlings were grown at 23 °C with 16 h of light at 60–100 μmol/m^2/s. Nine-day-old seedlings were collected and weighted.

2. Seedlings were homogenized with ice-cold Grinding buffer (tissue weight/buffer volume = 1 mg/3 µl).
   Note that the tissue and extract should be kept at 4 °C during all extraction processes.
3. Centrifuge at 16,000 *x g* at 4 °C for 10 min.
4. The supernatant is the resulting cellular extract, and the amount of protein was quantified by Bradford method (1976).

B. SOD activity staining

1. Proteins or cellular extract (15 to 25 µg) was subjected to 10% native-PAGE at 4 °C.
2. Wash the gel with distilled water for 3 times.
3. Incubate with NBT solution in dark with shaking for 15 min at room temperature (RT).
4. Pour off the NBT solution, wash the gel with distilled water for 3 times.
5. Incubate with Riboflavin solution in dark with shaking for 15 min at RT.
6. Pour off the Riboflavin solution, wash the gel with distilled water for 3 times.
7. Gel was illuminated with a white-light box for 10-15 min at RT. During illumination, immerse gel in a thin layer of distilled water to avoid drying the gel.
8. Under light exposure, the riboflavin is reduced then leading the production of O^{2-}. NBT is reduced by O^{2-} to form formazan, a dark blue color precipitate. The enriched SOD activity scavenges the O^{2-} to prevent the formation of formazan, thus, the white SOD activity bands appear in the blue background.

C. Identification of different SOD species (Figure 1)

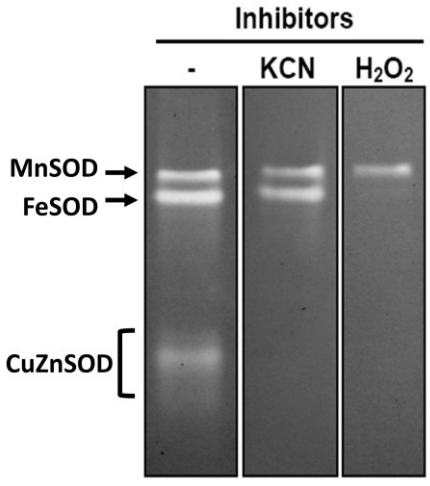

Figure 1. SOD activity verification in *Arabidopsis thaliana*. KCN is an inhibitor of CuZnSOD activity, whereas H_2O_2 inhibits both CuZnSOD and FeSOD activities. MnSOD activity is not inhibited by either treatment.

1. KCN treatment: KCN inhibits the CuZnSOD activity only.
   All procedures are the same as SOD activity staining processes except the addition of KCN to final 8 mM in Riboflavin solution.
2. H_2O_2 treatment: H_2O_2 inhibits both CuZnSOD and FeSOD activities.
   After native-PAGE and prior to NBT staining of SOD activity staining processes, soak the gel with 8 mM H_2O_2 solution for 30 min with shaking at room temperature. Wash the gel with distilled water for 3 times, and follow the remaining processes of SOD activity staining.

Recipes

1. Grinding buffer
   150 mM Tris (pH 7.2)
2. NBT solution
   0.1% NBT dissolved in distilled water.
   Store in 4 °C in dark
3. Riboflavin solution (freshly prepare before use)
   28 μM riboflavin and 28 mM TEMED in 0.1 M potassium phosphate buffer, pH 7.0.
4. 2 N KCN solution
   KCN in distilled water. Store in 4 °C.
5. 8 mM H_2O_2 solution (freshly prepare before use)
   Add 27 μl H_2O_2 (35%) into 30 ml 0.1 M potassium phosphate buffer, pH 7.0.

References

1. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
2. Kuo, W. Y., Huang, C. H., Liu, A. C., Cheng, C. P., Li, S. H., Chang, W. C., Weiss, C., Azem, A. and Jinn, T. L. (2013). CHAPERONIN 20 mediates iron superoxide dismutase (FeSOD) activity independent of its co-chaperonin role in *Arabidopsis* chloroplasts. *New Phytol* 197(1): 99-110.