SOD ACTIVITY ASSAY

(**Jiang and Zhang, 2002; Kant and Turan, 2011; Abedi and Pakniyat, 2010)**

The ability of SOD to inhibit the reduction of nitro blue tetrazolium (NBT) by $O2^-$ generated photochemically is measured according to the method of Beauchamp and Fridovich (1971), with slight modifications.

Extraction buffer

50 mM potassium phosphate buffer (pH 7,5)
1 mM EDTA
1% (w/v) PVP
1 mM PMSF

1. Frozen tissues is ground in liquid nitrogen with mortar and pestle.
2. Soluble proteins are extracted by homogenizing the powder in the appropriate extraction buffer (0,02g/1,5 ml).
3. The homogenate is centrifuged at 15, 000 g for 20 min at 4 °C and the supernatant is used for subesequent enzyme assays.

*active charcoal is added if necessary!

4. Protein content is determined according to the method of Bradford (1976) with BSA as standard.

Reaction buffer

50 mM phosphate buffer (pH 7.8)
13 mM Methionine
75 μM NBT
0,1 mM EDTA
100 μl of the enzyme extract
2 μM riboflavin

Total reaction volume: **1,5 ml**

- Riboflavin is added last and the tubes are placed below a light source consisting of two 15 W fluorescent lamps and the reaction allowed to run for 15 min.
- The reaction is stopped by switching-off the light and placing the tubes in the dark!
- The absorbance at 560 nm is read by a spectrophotometer.
- A non-irradiated reaction mixture which is run in parallel and which should not develop colour serves as **blank.**
- The reaction mixture lacking enzyme develops the maximum colour (**control**).
- The per cent inhibition at 560 nm is plotted as a function of volume of enzyme extract in the reaction mixture.
- **One enzyme unit (U)** of SOD activity is defined as the amount that inhibits the NBT photoreduction by 50% per minute.