

Detection of superoxide radical by EPR

BMPO

Product Code: B568

Spin trapping analysis is one of the most reliable techniques for detecting and identifying short-lived free radicals. The EPR (ESR) spin trap reagent detects both superoxide and hydroxyl radicals produced by systems *in vitro* and *in vivo*. BMPO was developed as a spin trapping reagent that adducts superoxide and shows a much longer half-life ($t_{1/2}$ =24 min) than other spin trap reagents. It gives us reproducible and steady results. Because BMPO is highly soluble in water, hydrophilic sample is applicable for analyzing the free radicals.

1. General Protocol

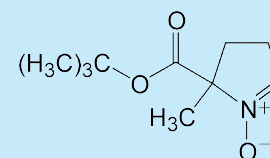
- ▶ Measuring hydroxy radical from Fenton reaction
 1. Add 15 μ l of BMPO solution, 75 μ l of 1 mM H_2O_2 and 75 μ l of 100 μ M $FeSO_4$ to 50 μ l of dd H_2O .
 2. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, e.g. 1 minute.
 3. Calculate relative intensity from the peak height.
- ▶ Measuring superoxide radical from xanthine oxidase(XO) reaction
 1. Dissolve 1 mg of BMPO with 1 ml of 50 mM Phosphate buffer(pH 7.4) (solution A).
 2. Prepare 50 mM Phosphate buffer(pH 7.4) containing 1 mM DTPA and 0.4 mM Xanthine(solution B).
 3. Prepare 50 mM Phosphate buffer(pH 7.4) containing 0.1 U/ml xanthine oxidase(solution C).
 4. Mix 15 μ l of solution A, 135 μ l of solution B and 10 μ l of solution C.
 5. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, e.g. 8 minutes.
 6. Calculate relative intensity from the peak height.

2. References

1. H. Zhao, J. Joseph, H. Zhang, H. Karoui and B. Kalyanaraman, *Free Radic Biol Med.* 2001;31:599-606.
2. G. M. Rosen, P. Tsai, J. Weaver, S. Porasuphatana, L. J. Roman, A. A. Starkov, G. Fiskum and S. Pou, *J Biol Chem.* 2002;277:40275-40280.

3. Specification

- ▶ Appearance: white crystal or crystalline powder
- ▶ Purity: $\geq 99.0\%$ (HPLC)



BMPO

5-*tert*-Butoxycarbonyl-5-methyl-1-pyrroline *N*-oxide
 $C_{10}H_{17}NO_3 = 199.25$
 Unit: 50 mg



Fig. 1 ESR Spectra of hydroxy radical adduct

(black: fenton reaction, blue: blank)

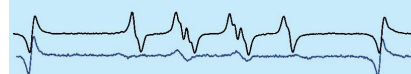


Fig. 2 ESR Spectra of superoxide radical adduct

(black: XO reaction, blue: blank)

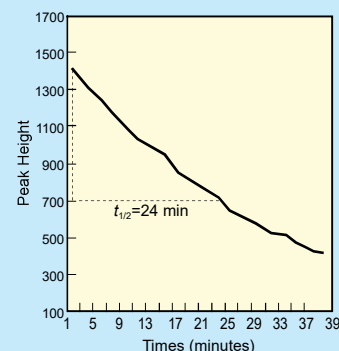


Fig. 3 Half-life of BMPO-superoxide radical adduct

1. Anti Oxidant Detection

2. DNA Damage Detection

3. Lipid Peroxide Detection

4. Radical Detection

5. Nitric Oxide Detection

6. NO Donor

7. AGEs Research