Detection of protein or DNA radical by WB, ELISA, and imaging

DMPO

Product Code: D048

Immuno-spin Trapping method was developed for detecting DNA and Protein radicals in biological analysis. ROS (Reactive Oxygen Species) produces modification of the structure and function of biomolecules that relate on the cause of variety diseases. To understand the mechanism of oxidative reactions, it is very important to analyze which molecules are involved in the oxidation process.
DMPO is the most popular spin-trapping reagent that traps radicals in protein and DNA samples. The DMPO-Protein or DMPO-DNA nitrone adducts are determined using a ELISA, Western Blotting, Mass Spectorometry, Imaging, and so on.
The purity of Dojindo's DMPO is higher than another commercialized DMPO. Since it does not contain impurities that might cause high background. Dojindo's DMPO does not require any pre-purification steps.

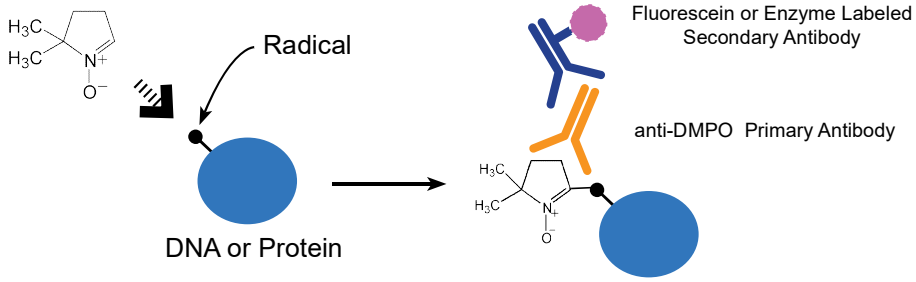


Fig. 1 Principle of Immuno-spin Trapping Method

1. Protocol Example : Radical DNA Detection

Referred Publication

Detection and imaging of the free radical DNA in cells--site-specific radical formation induced by Fenton chemistry and its repair in cellular DNA as seen by electron spin resonance, immuno-spin trapping and confocal microscopy. Bhattacharjee S, Chatterjee S, Jiang J, Sinha BK, Mason RP., *Nucleic Acids Res.* 2012, **40**, 5477-86

► Evaluation of radical DNA by ELISA

1. Treate RAW cells (1-3 x10^6 cells) with 100 µM $CuCl_2$,100 µM H_2O_2, and 100 mM DMPO and incubate for 12-15 hours at 5% CO_2 incubator.
2. Extract DNA from RAW cells and dilute DNA to 5 µg/ml in PBS.
3. Add 25µl of DNA solution and 25µl of Reacti-Bind DNA coating solution in each well of the plate and incubate for 2–4 hours at 37°C.
4. Wash the wells once with washing buffer (PBS containing 0.05% non-fat dry milk and 0.1% Tween-20).
5. Block with blocking buffer (PBS containing 3% non-fat dry milk) for 2 hours at 37°C.
6. Detect DMPO-DNA radical adduct with anti-DMPO antibody and HRP-conjugated secondary antibody.
7. After three washes, add the Immobilon chemiluminescence substrate each well and measure the intensity of luminesscense.

► Another application in this paper
Cell Imaging

2. Protocol Example : Radical Protein Detection

Referred Publication

Superoxide induces endothelial nitric-oxide synthase protein thiyl radical formation, a novel mechanism regulating eNOS function and coupling. Chen CA, Lin CH, Druhan LJ, Wang TY, Chen YR, Zweier JL., *J Biol Chem.* 2011, **286**,

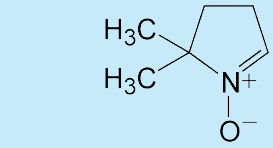
► Evaluation of radical protein by cell imaging

1. Prepare Bovine aortic endothelial cells (10^4 cells) in 35-mm dishes.
2. Add 10 µM Menadione and 50 mM DMPO and incubate cells.
3. Wash the cells with PBS and fix them with 3.7% paraformaldehyde for 10 minutes.
4. Permeabilize the cells with 0.25% Triton X-100 in TBST(Tris buffered saline with Tween) for 10 minutes and block the cells with 5% goat serum in TBST.
5. Visualize DMPO-protein radical adduct with anti-DMPO antibody and fluorescein labeled secondary antibody.
6. Analyze protein radicals by fluorescent microscopy.

► Other applications in this paper
Immunoblotting, Mass Spectrometry, Immunoprecipitation

3. Specification

► Appearance: colorless liquid
► Purity: ≥99.0%(GC)
► ESR spectrum: to pass test
► IR spectrum: authentic



DMPO
5,5-Dimethyl-1-pyrroline *N*-oxide
$C_6H_{11}NO$ = 113.16
CAS No. [3317-61-1]
Unit: 1 ml

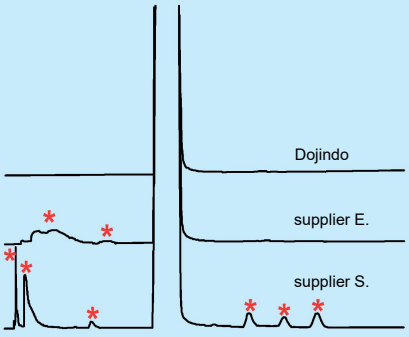


Fig. 2 Purity comparison in HPLC spectra
(*: impurities)

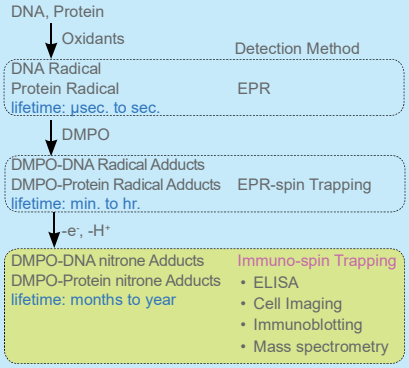


Fig. 3 Radical Detection Scheme

Detection of radical by EPR

DMPO

Product Code: D048

Because of potential cancer risks and their age-promoting effects, free radicals in living bodies have become a frequently studied subject. DMPO is the most frequently used spin-trapping reagent for the study of free radicals. It is suitable for trapping oxygen radicals, especially superoxides, and for producing adducts with characteristic EPR (ESR) patterns. However, most commercially available DMPO contains impurities that cause high backgrounds. Thus, DMPO requires further purification when running experiments on EPR. The quality of Dojindo's DMPO is well controlled and Dojindo's DMPO does not require any pre-purification process. There are no impurities to cause a background problem.

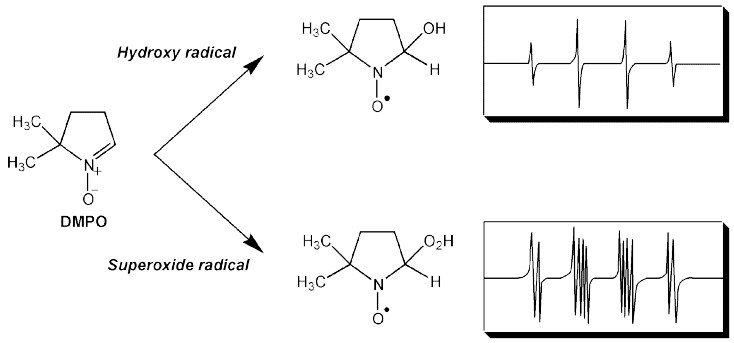
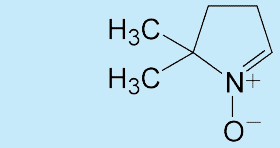


Fig. 1 ESR Spectera of DMPO Adducts



DMPO
5,5-Dimethyl-1-pyrroline *N*-oxide
$C_6H_{11}NO$ = 113.16
CAS No. [3317-61-1]
Unit: 1 ml

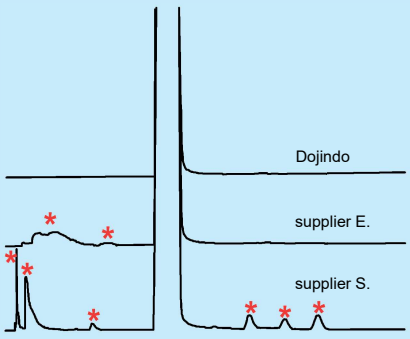


Fig. 2 Purity comparison in HPLC spectra
(*: impurities)

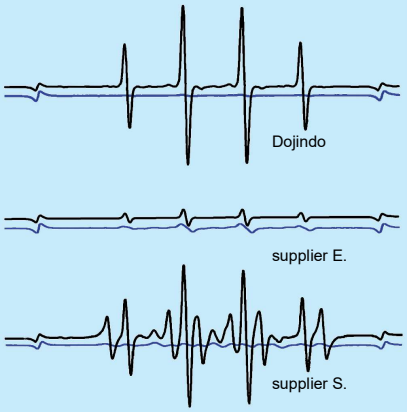


Fig. 3 Purity comparison of ESR spectra
(black: fenton reaction, blue: blank)

1. Protocol

► Evaluation of superoxide scavenging activities

1. Add 15 µl of DMPO and 50 µl of 5 mM hypoxanthine to 35 µl of 0.1 M Phosphate buffer(pH 7.8).
2. Add 50 µl of SOD standard or samples to be tested and voltex for 1-2 seconds.
3. Add 50 µl of 0.4 U/ml xanthine oxidase and voltex immediately.
4. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, e.g. 1 minute.
5. Calculate relative intensity(DMPO-O_2^-/Mn^{2+}) from the peak height.

► C-, N-, and S-centered radicals Detection

1. Prepare a solution of 100 mM phosphate buffer (pH 7.4) containing 25 µM DTPA.
2. Make up a solution of the following peroxidase substrates: (A) 100 mM sodium formate (HCOONa); (B) 100 mM potassium cyanide (KCN); (C) 100 mM sodium azide (NaN_3); (D) 100 mM sodium sulfite (Na_2SO_3) in 100 mM phosphate buffer, pH 7.4.
3. Make up a solution of horseradish peroxidase with concentration of 4.0 mg/ml (~ 100 µM) and 1 mM solution of hydrogen peroxide (H_2O_2).
4. Make up a solution of DMPO with concentration of 1 M.
5. Prepare your reaction mixture to a total reaction volume of 200 µl and add 130 µl of buffer to an Eppendorf tube.
6. Add 20 µl DMPO of your 1 M DMPO solution, 20 µl of one of the substrates' stock solutions, 10 µl of 1 mM H_2O_2, and initiate the reaction with 20 µl HRP.
7. Vortex the tube, transfer the solution to a flat cell, and acquire the spectrum.
8. The final concentrations of the components are: 100 mM DMPO, 10 mM substrate (formate, cyanide, azide, sulfite), 50 µM H_2O_2, and 10 µM HRP.

*This protocol was kindly provided by Bruker Corporation.

2. Specification

Refer to previous page (Page 15)

3. Recent Publications

Title	Reference
Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones	Yang Song, Brett A., and Garry R. Buettner, *et al., PNAS.* 2009; **106**: 9725 - 9730.
Manganese Superoxide Dismutase Modulates Hypoxia-Inducible Factor-1 Induction via Superoxide	Suwimol Kaewpila, and Larry W. Oberley, *et al., Cancer Res.* 2008; **68**: 2781 - 2788.
Hyperglycemia-Induced Reactive Oxygen Species Toxicity to Endothelial Cells Is Dependent on Paracrine Mediators	Julia V. Busik, and Maria B. Grant, *et al., Diabetes*, 2008; **57**: 1952 - 1965.
Overexpression of Extracellular Superoxide Dismutase Attenuates Heparanase Expression and Inhibits Breast Carcinoma Cell Growth and Invasion.	Melissa L.T., and Frederick E. Domann, *et al., Cancer Res.*, 2009; **69**: 6355 - 6363
Smoking Induces Bimodal DNA Damage in Mouse Lung	"Shunji Ueno and Kyosuke Temma, *Toxicol. Sci.* 2011; **120**: 322 - 330. "
Cardiac Myocyte–Specific Expression of Inducible Nitric Oxide Synthase Protects Against Ischemia/Reperfusion Injury by Preventing Mitochondrial Permeability Transition	Matthew B. and Aruni Bhatnagar, *et al., Circulation*. 2008; **118**: 1970 - 1978.