

# IMMUNO-SPIN TRAPPING

**DMPO, pAb**

**ALX-210-530-R050**

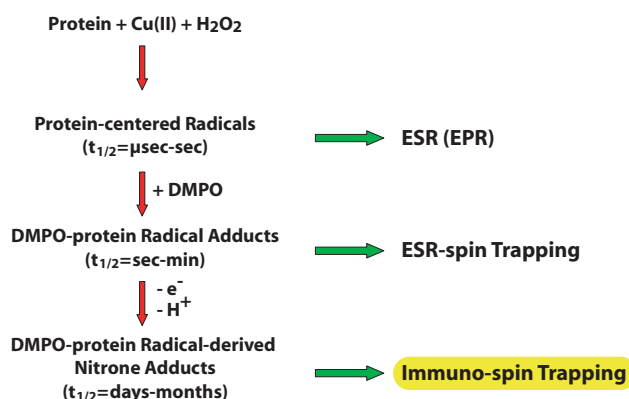
**50  $\mu$ l**

## Introduction

Detection of protein-derived radicals in isolated proteins has typically required the utilization of direct electron spin resonance (ESR) and/or spin trapping ESR. ESR can be applied both to understand the mechanisms of protein radical formation and for structural identification of the amino acid-derived radical involved. However, ESR measurements require significant levels of protein and of its derived radicals (greater than micromolar) and rely on rather specialized and costly equipment. Moreover, detection of protein radicals by ESR becomes a difficult task in cells and tissues.

Immuno-spin trapping is based on the fact that certain amino acid-derived radicals (most notably, but not exclusively, tyrosyl radicals) can react with the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) to form a protein-DMPO nitroxide radical adduct, which can then be oxidized by one electron to the corresponding ESR-silent protein-DMPO nitron adduct. Indeed, DMPO spin adducts are not stable, have a half-life on the order of minutes, and tend to decay under oxidizing conditions to the rather stable DMPO nitron. Rabbit polyclonal anti-DMPO nitron antiserum (Prod. No. ALX-210-530) has been developed and validated in Mason's laboratory (C. D. Detweiler, L. J. Deterding, K. B. Tomer, C. F. Chignell, D. Germolec, R. P. Mason: *Immunological identification of the heart myoglobin radical formed by hydrogen peroxide*; Free Radic. Biol. Med. **33**, 364 (2002)) and a series of studies have shown its utility to demonstrate protein radical formation in different proteins exposed to a variety of oxidants.

In summary, immuno-spin trapping is proving to be a potent, sensitive, and accessible method to detect low levels (e.g. greater than nanomolar) of protein-derived radicals produced *in vitro* and potentially, and yet to be established, *in vivo*. Moreover, it has just been established that anti-DMPO nitron antibodies can be utilized for the detection of DNA-derived radicals in the form of DNA-DMPO nitron adducts (D. C. Ramirez, S. E. Mejiba, R.



**FIGURE:** Schematic overview of approaches to detect protein radicals.

P. Mason: *Immuno-spin trapping of DNA radicals*; Nat. Methods **3**, 127 (2006)), which broadens even more the scope of applications of this breakthrough technique in free radical biology and medicine.

Adapted from: *Immuno-spin trapping: A breakthrough for the sensitive detection of protein-derived radicals, a commentary on "Protein radical formation on thyroid peroxidase during turnover"*; R. Radi; Free Radic. Biol. Med. **41**, 416 (2006).

## Selected Review Article

Using anti-5,5-dimethyl-1-pyrroline N-oxide (anti-DMPO) to detect protein radicals in time and space with immuno-spin trapping: R. P. Mason; Free Radic. Biol. Med. **36**, 1214 (2004)

## DMPO, pAb

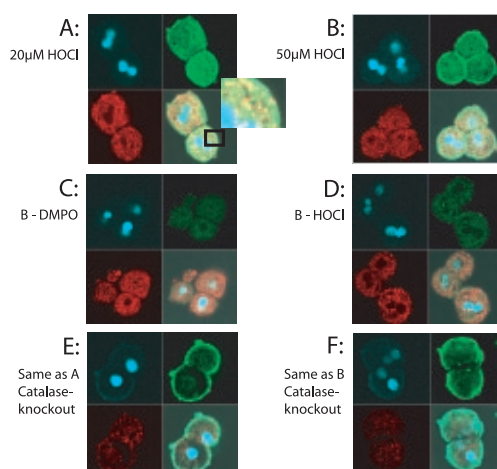
ALX-210-530-R050

50 µl

From rabbit. **IMMUNOGEN:** DMPO-octanoic acid conjugated to ovalbumin. **SPECIFICITY:** Recognizes DMPO (Prod. No. ALX-430-090), DMPO-octanoic acid and DMPO-protein adducts. Does not cross react with non-adducted proteins. **APPLICATION:** ELISA, IHC, WB.

### Widely Published

**LIT:** Immunological identification of the heart myoglobin radical formed by hydrogen peroxide: C. D. Detweiler, et al.; *Free Radic. Biol. Med.* **33**, 364 (2002) • Immunological detection of hemoglobin-derived radicals formed by reaction with hydrogen peroxide: involvement of a protein-tyrosyl radical: D. C. Ramirez, et al.; *Free Radic. Biol. Med.* **34**, 830 (2003) • Identification of free radicals on hemoglobin from its self-peroxidation using mass spectrometry and immuno-spin trapping: observation of a histidinyl radical: L. Deterding; *J. Biol. Chem.* **279**, 11600 (2004) • Protein radical formation during lactoperoxidase-mediated oxidation of the suicide substrate glutathione: immunochemical detection of a lactoperoxidase radical-derived 5,5-dimethyl-1-pyrroline N-oxide nitron adduct: Q. Guo, et al.; *J. Biol. Chem.* **279**, 13272 (2004) • Mechanism of hydrogen peroxide-induced Cu,Zn-superoxide dismutase-centered radical formation as explored by immuno-spin trapping: the role of copper- and carbonate radical anion-mediated oxidations: D. C. Ramirez, et al.; *Free Radic. Biol. Med.* **38**, 201 (2005) • Identification of the myoglobin tyrosyl radical by immuno-spin trapping and its dimerization: C. D. Detweiler, et al.; *Free Radic. Biol. Med.* **38**, 969 (2005) • Immuno-spin trapping: detection of protein-centered radicals: D. C. Ramirez, et al.; *Curr. Prot. Toxicol.* **2**, 17.7.1 (2005) • Superoxide generation from mitochondrial NADH dehydrogenase induces self-inactivation with specific protein radical formation: Y. R. Chen, et al.; *J. Biol. Chem.* **280**, 37339 (2005) • Immunochemical detection of nitric oxide and nitrogen dioxide trapping of the tyrosyl radical and the resulting nitrotyrosine in sperm whale myoglobin: K. Nakai and R. P. Mason; *Free Radic. Biol. Med.* **39**, 1050 (2005) • Immuno-spin trapping of DNA radicals: D. C. Ramirez, et al.; *Nat. Methods* **3**, 123 (2006) • Immuno-spin trapping of hemoglobin and myoglobin radicals derived from nitrite-mediated oxidation: A. Keszler, et al.; *Free Radic. Biol. Med.* **40**, 507 (2006) • Protein radical formation on thyroid peroxidase during turnover: M. Ehrenshaft, et al.; *Free Radic. Biol. Med.* **41**, 422 (2006) • Protein radical formation on thyroid peroxidase during turnover: M. Ehrenshaft, et al.; *Free Radic. Biol. Med.* **41**, 422 (2006) • Immunolocalization of hypochlorite-induced, catalase-bound free radical formation in mouse hepatocytes: M. G. Bonini et al.; *Free Rad. Biol. Med.* **42**, 530 (2007) • Mitochondrial Complex II in the Post-ischemic Heart: Oxidative injury and the role of protein S-Glutathionylation: Y. R. Chen, et al.; *J. Biol. Chem.* **282**, 32640 (2007) • Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants: P. Cassina, et al.; *J. Neurosci.* **28**, 4115 (2008) • Direct evidence of iNOS-mediated in vivo free radical production and protein oxidation in acetone-induced ketosis: K. Stadler, et al.; *Am. J. Phys. Endocrin. Metab.* **295**, E456 (2008) • Immuno-spin trapping of a post-translational carboxypeptidase B1 radical formed by a dual role of xanthine oxidase and endothelial nitric oxide synthase in acute septic mice: S. Chatterjee, et al.; *Free Radic. Biol. Med.* **46**, 454 (2009) • Immuno-spin trapping of protein and DNA radicals: "tagging" free radicals to locate and understand the redox process: S. E. Gomez-Mejiba, et al.; *Free Radic. Biol. Med.* **46**, 853 (2009)



**FIGURE:** Confocal microscopy images of the colocalization of catalase (red stain) and protein-DMPO adducts (green stain) obtained by treating mouse hepatocytes (2.5x10<sup>6</sup> cells/ml) with H<sub>2</sub>O<sub>2</sub>.

Picture courtesy of Dr. M. G. Bonini, National Institute of Environmental Health Sciences, Research Triangle Park (RTP), NC, USA.

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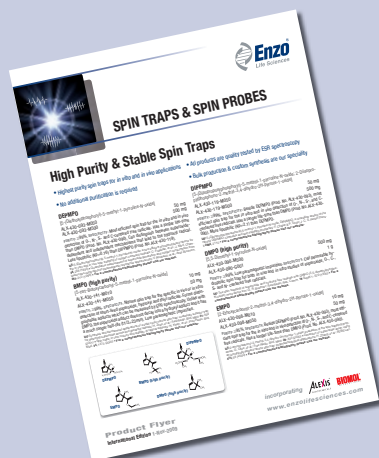
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## Application Note

### Immuno-spin Trapping of DNA Radicals

The detection of DNA radicals by immuno-spin Trapping (IST) is based on the trapping of radicals with 5,5-dimethyl-1-pyrroline N-oxide (DMPO), forming stable nitron adducts that are then detected using an anti-DMPO serum. IST combines the simplicity, reliability, specificity and sensitivity of spin trapping with heterogeneous immunoassays for the detection of DNA radicals, and complements existing methods for the measurement of oxidatively generated DNA damage.

**LIT:** Immuno-spin trapping of DNA radicals: D. C. Ramirez, et al.; *Nat. Methods* **3**, 123 (2006) • Immuno-spin trapping analyses of DNA radicals: D. C. Ramirez, et al.; *Nat. Protoc.* **2**, 512 (2007) • Immuno-spin trapping of protein and DNA radicals: "tagging" free radicals to locate and understand the redox process: S. E. Gomez-Mejiba, et al.; *Free Radic. Biol. Med.* **46**, 853 (2009)



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