

Spin Traps & Spin Probes

includes Immuno-spin Trapping

highlight

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High Purity & Stable Spin Traps

- Highest purity spin traps for *in vitro* and *in vivo* applications.
- No additional purification is required.
- All products are quality tested by ESR spectroscopy.
- Bulk production & custom synthesis are our speciality.

NEW

BMPO (high purity)

[5-*tert*-Butoxycarbonyl-5-methyl-1-pyrroline-N-oxide (high purity)]

ALX-430-141-M010

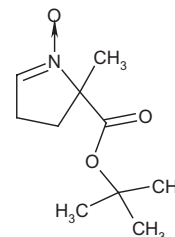
10 mg

ALX-430-141-M050

50 mg

Nitrone spin trap for the specific *in vivo* or *in vitro* detection of short-lived superoxide, hydroxyl and thyl radicals. Forms distinguishable adducts which can be measured by EPR spectroscopy. Unlike with DMPO, the superoxide adduct does not decay into a hydroxyl adduct and it has a much longer half-life ($t_{1/2}$ =23min). Low paramagnetic impurities. **No further purification required.**

LIT: Synthesis and biochemical applications of a solid cyclic nitrone spin trap: a relatively superior trap for detecting superoxide anions and glutathionyl radicals: H. Zhao, et al.; Free Radic. Biol. Med. **31**, 599 (2001) • Spin traps: in vitro toxicity and stability of radical adducts: N. Khan, et al.; Free Radic. Biol. Med. **34**, 1473 (2003) • Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence: H. Zhao, et al.; PNAS **102**, 5727 (2005)



DEPMPO

[5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide]

ALX-430-093-M050

50 mg

ALX-430-093-M500

500 mg

PURITY: ≥99%. **SPECIFICITY:** Most efficient spin trap for the *in vitro* and *in vivo* detection of O $\cdot$ -, N $\cdot$ - and C-centered free radicals. Has a longer life-time than DMPO (Prod. No. ALX-430-090). Can distinguish between superoxide-dependent and independent mechanisms that lead to the hydroxyl radical. Less lipophilic (K_p =0.16) than DIPPMPPO (Prod. No. ALX-430-119). See Figure 1 and 2.

LIT: 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide: a new efficient phosphorylated nitrone for the *in vitro* and *in vivo* spin trapping of oxygen-centered radicals: C. Frejaville, et al.; J. Med. Chem. **38**, 258 (1995) • Quantitative measurement of superoxide generation and oxygen consumption from leukocytes using electron paramagnetic resonance spectroscopy: V. Roubaud, et al.; Anal. Biochem. **257**, 210 (1998) • Evaluation of DEPMPO as a spin trapping agent in biological systems: K.J. Liu, et al.; Free Radic. Biol. Med. **26**, 714 (1999) • For a comprehensive bibliography please visit our website.

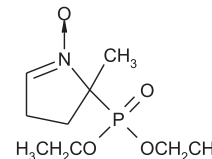


FIGURE 1:
Spectrum of the
(O $_2^{\cdot-}$)-DEPMPO
adduct.



FIGURE 2:
Spectrum of the
(HO)-DEPMPO
adduct.

ALEXIS
BIOCHEMICALS

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High Purity & Stable Spin Traps

continued

DIPPMPO

[5-(Diisopropoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide; 2-Diisopropylphosphono-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide]

ALX-430-119-M050 50 mg

ALX-430-119-M500 500 mg

PURITY: ≥99%. **SPECIFICITY:** Beside DEPMPO (Prod. No. ALX-430-093), most efficient spin trap for the *in vitro* and *in vivo* detection of O-, N-, S-, and C-centered free radicals. Has a longer life-time than DMPO (Prod. No. ALX-430-090). More lipophilic (K_p=2.1) than DEPMPO.

LIT: 5-(Diisopropoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide, DIPPMPO, a crystalline analog of the nitron DEPMPO: synthesis and spin trapping properties: F. Chaler & P. Tordo; J. C. S. Perkin Trans. II **2002**, 2110. The line asymmetry of electron spin resonance spectra as a tool to determine the cis/trans ratio for spin-trapping adducts of chiral pyrrolines N-oxides: the mechanism of formation of hydroxyl radical adducts of EMPO, DEPMPO, and DIPPMPO: M. Culcasi, et al; Free Radic. Biol. Med. **40**, 1524 (2006)

DMPO (high purity)

[5,5-Dimethyl-1-pyrroline-N-oxide]

ALX-430-090-M500 500 mg

ALX-430-090-G001 1 g

PURITY: ≥99%. Low paramagnetic impurities. **SPECIFICITY:** Cell permeable hydrophilic spin trap for both *in vivo* and *in vitro* studies of superoxide, O-, C-, S- and N- centered free radicals. **No further purification required.**

LIT: The spin trapping of superoxide and hydroxyl free radicals with DMPO (5,5-dimethylpyrroline-N-oxide): more about iron: G.R. Buettner; Free Radic. Res. Comm. **19** Suppl 1, 579 (1993). For a comprehensive bibliography please visit our website.

EMPO

[2-Ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide]

ALX-430-098-M010 10 mg

ALX-430-098-M050 50 mg

PURITY: ≥95%. **SPECIFICITY:** Beside DEPMPO (Prod. No. ALX-430-093), most efficient spin trap for the *in vitro* and *in vivo* detection of O-, N-, S-, and C-centered free radicals. Has a longer life-time than DMPO (Prod. No. ALX-430-090). See Figure 3.

LIT: 2-ethoxycarbonyl-2-methyl-3,4-dihydro-2H-pyrrole-1-oxide: evaluation of the spin trapping properties: G. Olive, et al; Free Radic. Biol. Med. **28**, 403 (2000). Detection of superoxide anion using an isotopically labeled nitron spin trap: potential biological applications: H. Zhang, et al; FEBS Lett. **473**, 58 (2000). Kinetic study and theoretical analysis of hydroxyl radical trapping and spin adduct decay of alkoxy carbonyl and dialkoxyphosphoryl nitrones in aqueous media: F.A. Villamena, et al; J. Phys. Chem. (A) **107**, 4407 (2003). Spin adduct formation from lipophilic EMPO-derived spin traps with various oxygen- and carbon-centered radicals: K. Stolze, et al; Biochem. Pharmacol. **69**, 297 (2005). For a comprehensive bibliography please visit our website.

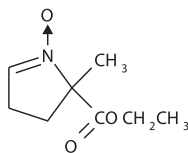
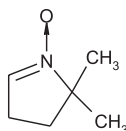
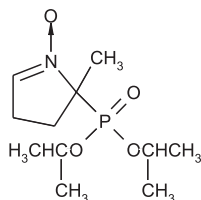


FIGURE 3:
Spectrum of the
(O₂)-EMPO
adduct.

Selected Literature
References of Newly
Described Spin Traps &
Spin Probes**MPPO**

Studies on the stability of oxygen radical spin adducts of a new spin trap: 5-methyl-5-phenylpyrroline-1-oxide (MPPO): N. Sankuratri, et al; Free Radic. Biol. Med. **21**, 889 (1996)

5-ChEPMPPO

Synthesis and spin-trapping behavior of 5-ChEPMPPO, a cholesteryl ester analogue of the spin trap DEPMPO: M. Hardy, et al; J. Org. Chem. **70**, 10426 (2005)

3,5-EDPO

Very stable superoxide radical adducts of 5-ethoxycarbonyl-3,5-dimethyl-pyrroline N-oxide (3,5-EDPO) and its derivatives: K. Stolze, et al; Biochem. Pharmacol. **69**, 1351 (2005)

4-PhenylDEPMPO

Diastereoselective synthesis and ESR study of 4-phenylDEPMPO spin traps: M. Hardy, et al; J. Org. Chem. **70**, 2135 (2005)

Ester Nitrones DEEPN & EPPyON

Preparation and use as spin trapping agents of new ester-nitrones: A. Allouch, et al; Org. Biomol. Chem. **1**, 593 (2003)

Spin trapping of superoxide by diester-nitrones: A. Allouch, et al; Org. Biomol. Chem. **3**, 2458 (2005)

AMPO

Spin trapping by 5-carbamoyl-5-methyl-1-pyrroline N-oxide (AMPO): theoretical and experimental studies: F.A. Villamena, et al; J. Org. Chem. **69**, 7994 (2004)

Series of EMPO Derivatives

Spin adduct formation from lipophilic EMPO-derived spin traps with various oxygen- and carbon-centered radicals: K. Stolze, et al; Biochem. Pharmacol. **69**, 297 (2005)

Spin trapping of C- and O-centered radicals with methyl-, ethyl-, pentyl-, and phenyl-substituted EMPO derivatives: K. Stolze, et al; Bioorg. Med. Chem. **14**, 3368 (2006)

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Technical Note

Stability and *In Vitro* Toxicity of Radical Adducts

The radical adducts of the new spin traps DEPMPO and EMPO have significantly increased stability as compared to DMPO. This indicates that the new spin traps potentially offer increased stability of spin adducts in functioning cells. As there are some effects on cells from these spin traps, each type of *in vivo* application is likely to need preliminary studies of stability and toxicity to determine which spin trap is the most appropriate for the experimental goal.

LIT: Spin traps: *in vitro* toxicity and stability of radical adducts: N. Khan, et al; Free Radic. Biol. Med. **34**, 1473 (2003). Cytotoxicity of novel derivatives of the spin trap EMPO: N. Rohr-Udilova, et al; Bioorg. Med. Chem. Lett. **16**, 541 (2006)

Spin Traps & Spin Probes
Selected Latest Review Articles

Unique *in vivo* applications of spin traps: L.J. Berliner, et al; Free Radic. Biol. Med. **30**, 489 (2001)

Measurements *in vivo* of parameters pertinent to ROS/RNS using EPR spectroscopy: N. Khan & H. Swartz; Mol. Cell Biochem. **234**, 341 (2002)

In vivo electron spin resonance-computed tomography/nitroxyl probe technique for non-invasive analysis of oxidative injuries: H. Utsumi & K. Yamada; Arch. Biochem. Biophys. **416**, 1 (2003)

Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean?: B. Halliwell & M. Whiteman; Br. J. Pharmacol. **142**, 231 (2004)

In vivo EPR spectroscopy: biomedical and potential diagnostic applications: S.K. Jackson, et al; Faraday Discuss. **126**, 103 (2004)

In vivo spin trapping of nitric oxide: L.J. Berliner & H. Fujii; Antioxid. Redox Signal. **6**, 649 (2004)

In vitro and *in vivo* measurement of pH and thiols by EPR-based techniques: V.V. Khramtsov, et al; Antioxid. Redox Signal. **6**, 667 (2004)

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)

Recent progress in *in vivo* ESR spectroscopy: K. Takeshita & T. Ozawa; J. Radiat. Res. (Tokyo) **45**, 373 (2004)

Nitron spin on cerebral ischemia: S.A. Doggrell; Curr. Opin. Investig. Drugs **7**, 20 (2006)

Use of spectroscopic probes for detection of reactive oxygen species: G. Bartosz; Clin. Chim. Acta **368**, 53 (2006)

Why to use cyclic hydroxylamine spin probes?

Comparison of spin traps and spin probes.

Spin trapping is widely used for unambiguous detection of free radicals, such as superoxide radical. Unfortunately, EPR detection of the $O_2^{\cdot-}$ radicals in biological systems is limited by slow kinetics of $O_2^{\cdot-}$ spin trapping ($\sim 55 \text{ M}^{-1}\text{s}^{-1}$) and biodegradation of the radical adducts (reduction to EPR silent hydroxylamines and oxidation to secondary nitrones) [1]. It has been previously shown that PP-H is an effective scavenger of the $O_2^{\cdot-}$ [2], rapidly reacts with $O_2^{\cdot-}$ to form stable nitroxides with a much longer half-life than $O_2^{\cdot-}$ radical adducts. This is a distinct advantage of cyclic hydroxylamines over nitron spin traps, which form relatively stable $O_2^{\cdot-}$ adducts in cell-free systems [3], but these adducts

rapidly degrade in biological samples [4]. Cyclic hydroxylamines react with $O_2^{\cdot-}$ 100 times faster ($\sim 104 \text{ M}^{-1}\text{s}^{-1}$) than spin traps, which allow cyclic hydroxylamines to compete with cellular antioxidants and react with intracellular $O_2^{\cdot-}$. The lack of specificity of cyclic hydroxylamines can be overcome by use of superoxide dismutases or inhibitors of $O_2^{\cdot-}$ production by NADPH oxidase, uncoupled eNOS (NOSIII), xanthine oxidase, apocynin, L-NAME, and oxypurinol [5-7]. Cationic, anionic and neutral spin probes with various lipophilicity and cell permeability allow site-specific $O_2^{\cdot-}$ detection with higher sensitivity than nitron spin traps.

LIT: [1] Inhibition of radical adduct reduction and reoxidation of the corresponding hydroxylamines in in vivo spin trapping of carbon tetrachloride-derived radicals: M. Sentjurc & R. P. Mason; Free Radic. Biol. Med. **13**, 151 (1992) ■ [2] Noninvasive diagnostic tool for inflammation-induced oxidative stress using electron spin resonance spectroscopy and an extracellular cyclic hydroxylamine: S. I. Dikalov, et al.; Arch. Biochem. Biophys. **402**, 218 (2002) ■ [3] Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence: H. Zhao, et al.; PNAS **102**, 5727 (2005) ■ [4] Oxygen radical generation and enzymatic properties of mitochondria in hypoxia/reoxygenation: K. Zwicker, et al.; Arzneimittelforschung **48**, 629 (1998) ■ [5] Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase: N. Kuzkaya, et al.; Biochem. Pharmacol. **70**, 343 (2005) ■ [6] C242T CYBA polymorphism of the NADPH oxidase is associated with reduced respiratory burst in human neutrophils: K. E. Wyche, et al.; Hypertension **43**, 1246 (2004) ■ [7] Regulation of xanthine oxidoreductase protein expression by hydrogen peroxide and calcium: J. S. McNally, et al.; Arterioscler. Thromb. Vasc. Biol. **25**, 1623 (2005)

Type	Name	Structure	Lipophilicity	Lifetime of O ₂ ^{•-} Products	Detection	Applications	Key Literature
Spin Traps	PBN		10	–	R [•] RO [•]	Detection of R-C [•] <i>in vitro</i> and <i>in vivo</i> .	[1] Spin-trapping methods for detecting superoxide and hydroxyl free radicals in vitro and in vivo: G. R. Buettner & R. P. Mason; Meth. Enzymol. 186 , 127 (1990) [2] In vivo evidence of hydroxyl radical formation after acute copper and ascorbic acid intake: electron spin resonance spin-trapping investigation: M. B. Kadiiska, et al.; Mol. Pharmacol. 42 , 723 (1992)
	POBN		0.15	–			[3] 5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide: a new efficient phosphorylated nitron for the in vitro and in vivo spin trapping of oxygen-centered radicals: C. Frejaville, et al.; J. Med. Chem. 38 , 258 (1995)
	DMPD		0.06	0.9 min	OH [•] , O ₂ ^{•-} , R [•] , RO [•]	Cell-free and extracellular detection of OH [•] , O ₂ ^{•-} , R [•] , S [•] .	[4] Detection of superoxide anion using an isotopically labeled nitron spin trap: potential biological applications: H. Zhang, et al.; FEBS Lett. 473 , 58 (2000) [5] Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence: H. Zhao, et al.; PNAS 102 , 5727 (2005) [6] Synthesis and biochemical applications of a solid cyclic nitron spin trap: a relatively superior trap for detecting superoxide anions and glutathionyl radicals: H. Zhao, et al.; Free Radic. Biol. Med. 31 , 599 (2001) [7] Spin traps: in vitro toxicity and stability of radical adducts: N. Khan, et al.; Free Radic. Biol. Med. 34 , 1473 (2003)
	EMPO		0.33	10 min	OH [•] O ₂ ^{•-} RO ₂ [•] R [•] S [•]		
	DEPMPO		0.16	17 min			
	BMPO		–	23 min	OH [•] , O ₂ ^{•-} , S [•]		
Cyclic Hydroxylamines	PP-H		0.005	More than 4 hours in blood plasma, in cells and tissue	O ₂ ^{•-} ONOOH Aryl-O [•]	Quantitative measurements of extracellular O ₂ ^{•-} and quantification of intracellular O ₂ ^{•-} in cells and tissue samples.	[8] Spin trapping of superoxide radicals and peroxynitrite by 1-hydroxy-3-carboxy-pyrrolidine and 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine and the stability of corresponding nitroxyl radicals towards biological reductants: S. Dikalov, et al.; BBRC 231 , 701 (1997) [9] Comparison of glyceryl trinitrate-induced with pentaerythritol tetranitrate-induced in vivo formation of superoxide radicals: effect of vitamin C: S. Dikalov, et al.; Free Radic. Biol. Med. 27 , 170 (1999) [10] Measurement of oxidative stress by EPR radical-probe technique: L. Valgimigli, et al.; Free Radic. Biol. Med. 31 , 708 (2001) [11] Noninvasive diagnostic tool for inflammation-induced oxidative stress using electron spin resonance spectroscopy and an extracellular cyclic hydroxylamine: S. I. Dikalov, et al.; Arch. Biochem. Biophys. 402 , 218 (2002)
	CAT1-H		0.01				[12] Pharmacokinetic study of acyl-protected hydroxylamine probe, 1-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrrolidine, for in vivo measurements of reactive oxygen species: K. Saito, et al.; Free Radic. Biol. Med. 36 , 517 (2004)
	CP-H		0.05				[13] Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase: N. Kuzkaya, et al.; Biochem. Pharmacol. 70 , 343 (2005)
	CM-H		27	Can be used in vivo i.p. and i.v. injections		In vivo O ₂ ^{•-} detection.	[14] Epr analysis reveals three tissues responding to endotoxin by increased formation of reactive oxygen and nitrogen species: A. V. Kozlov, et al.; Free Radic. Biol. Med. 34 , 1555 (2003) [15] Role of extracellular superoxide dismutase in hypertension: M.C. Gongora, et al.; Hypertension 48 , 473 (2006)
	TMT-H		35				
	TM-H		43				

Cyclic Hydroxylamine Spin Probes

CAT1-H · HCl

[1-Hydroxy-2,2,6,6-tetramethylpiperidin-4-yl-trimethylammonium chloride]

ALX-430-131-M010	10 mg
ALX-430-131-M050	50 mg
ALX-430-131-M250	250 mg

Cyclic hydroxylamine spin probe for *in vivo*, intraperitoneal and intravenous injections. Lifetime of more than 4 hours in blood plasma, in cells and tissue. Allows quantitative measurements of extracellular $O_2^{\cdot-}$ and quantification of intracellular $O_2^{\cdot-}$ in cells and tissue samples and *in vivo* $O_2^{\cdot-}$ detection.

LIT: Role of extracellular superoxide dismutase in hypertension: M.C. Gongora, et al.; Hypertension **48**, 473 (2006)

CM-H · HCl

[1-Hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine]

ALX-430-117-M010	10 mg
ALX-430-117-M050	50 mg
ALX-430-117-M250	250 mg

Analog of CP-H (Prod. No. ALX-430-078) with higher cell permeability and 3-fold better reactivity with superoxide. Has been used to study intracellular production of superoxide radicals in plasma, endothelial cells and isolated hearts.

LIT: Detection of superoxide with new cyclic hydroxylamine CMH in plasma, cells and isolated heart: B. Fink & S. Dikalov; Free Radic. Biol. Med. **33**, S366 (2002) • Oscillatory shear stress stimulates endothelial production of $O_2^{\cdot-}$ from p47phox-dependent NAD(P)H oxidases, leading to monocyte adhesion: J. Hwang, et al.; J. Biol. Chem. **278**, 47291 (2003) • Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase: N. Kuzkaya, et al.; Biochem. Pharmacol. **70**, 343 (2005) • Nox1 overexpression potentiates angiotensin II-induced hypertension and vascular smooth muscle hypertrophy in transgenic mice: A. Dikalova, et al.; Circulation **112**, 2668 (2005)

CP-H · HCl

[1-Hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine]

ALX-430-078-M010	10 mg
ALX-430-078-M050	50 mg
ALX-430-078-M250	250 mg

Effective, cell permeable and non-toxic spin trap for the detection of superoxide radical and peroxynitrite, both *in vitro* and *in vivo*. Resistant to reduction by vitamin C and thiols.

LIT: Spin trapping of superoxide radicals and peroxynitrite by 1-hydroxy-3-carboxy-pyrrolidine and 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine and the stability of corresponding nitroxyl radicals towards biological reductants: S. Dikalov, et al.; BBRC **231**, 701 (1997) • Quantification of superoxide radicals and peroxynitrite in vascular cells using oxidation of sterically hindered hydroxylamines and electron spin resonance: S. Dikalov, et al.; Nitric Oxide **1**, 423 (1997) • Comparison of glycyl trinitrate-induced with pentaerythritol trinitrate-induced in vivo formation of superoxide radicals: effect of vitamin C: S. Dikalov, et al.; Free Radic. Biol. Med. **27**, 170 (1999)

• A new approach for extracellular spin trapping of nitroglycerin-induced superoxide radicals both *in vitro* and *in vivo*: B. Fink, et al.; Free Radic. Biol. Med. **28**, 121 (2000) • Noninvasive diagnostic tool for inflammation-induced oxidative stress using electron spin resonance spectroscopy and an extracellular cyclic hydroxylamine: S.I. Dikalov, et al.; Arch. Biochem. Biophys. **402**, 218 (2002) • Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric oxide synthase: N. Kuzkaya, et al.; J. Biol. Chem. **278**, 22546 (2003) • Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase: N. Kuzkaya, et al.; Biochem. Pharmacol. **70**, 343 (2005)

PP-H · HCl

[1-Hydroxy-4-phosphono-oxy-2,2,6,6-tetramethylpiperidine]

ALX-430-080-M010	10 mg
ALX-430-080-M050	50 mg

Effective, non-cell permeable and non-toxic analog of CP-H, for the detection of superoxide radical. Resistant against reduction of vitamin C. For the cell permeable analog see CP-H (Prod. No. ALX-430-078).

LIT: Detection of superoxide radicals and peroxynitrite by 1-hydroxy-4-phosphonoxy-2,2,6,6-tetramethylpiperidine: quantification of extracellular superoxide radicals formation: S. Dikalov, et al.; BBRC **248**, 211 (1998) • A new approach for extracellular spin trapping of nitroglycerin-induced superoxide radicals both *in vitro* and *in vivo*: B. Fink, et al.; Free Radic. Biol. Med. **28**, 121 (2000) • Noninvasive diagnostic tool for inflammation-induced oxidative stress using electron spin resonance spectroscopy and an extracellular cyclic hydroxylamine: S.I. Dikalov, et al.; Arch. Biochem. Biophys. **402**, 218 (2002)

TM-H · HCl

[1-Hydroxy-4-methoxy-2,2,6,6-tetramethylpiperidine]

ALX-430-132-M010	10 mg
ALX-430-132-M050	50 mg
ALX-430-132-M250	250 mg

Cyclic hydroxylamine spin probe for *in vivo*, intraperitoneal and intravenous injections. Lifetime of more than 4 hours in blood plasma, in cells and tissue. Allows quantitative measurements of extracellular $O_2^{\cdot-}$ and quantification of intracellular $O_2^{\cdot-}$ in cells and tissue samples and *in vivo* $O_2^{\cdot-}$ detection.

TMT-H · HCl

[N-(1-Hydroxy-2,2,6,6-tetramethylpiperidin-4-yl)-2-methylpropanamide]

ALX-430-133-M010	10 mg
ALX-430-133-M050	50 mg
ALX-430-133-M250	250 mg

Cyclic hydroxylamine spin probe for *in vivo*, intraperitoneal and intravenous injections. Lifetime of more than 4 hours in blood plasma, in cells and tissue. Allows quantitative measurements of extracellular $O_2^{\cdot-}$ and quantification of intracellular $O_2^{\cdot-}$ in cells and tissue samples and *in vivo* detection.

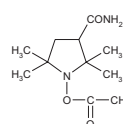
Probe for the Detection of ROS

ACP

ALX-430-118-M010	10 mg
ALX-430-118-M050	50 mg
ALX-430-118-M250	250 mg

Cell permeable probe for the detection of intracellular oxidative stress. ACP can easily be deprotected with esterases to cyclic hydroxylamines, which are oxidized by ROS leading to ESR-detectable nitroxide radicals.

LIT: Sensitive ESR determination of intracellular oxidative stress by using acyl-protected hydroxylamines as new spin reagents: O. Itoh, et al.; Chem. Lett. **2000**, 304 • *In vivo* EPR imaging by using an acyl-protected hydroxylamine to analyze intracerebral oxidative stress in rats after epileptic seizures: H. Yokoyama, et al.; Magn. Reson. Imaging **18**, 875 (2000) • Measurement of oxidative stress by EPR radical-probe technique: L. Valgimigli, et al.; Free Radic. Biol. Med. **31**, 708 (2001) • Pharmacokinetic study of acyl-protected hydroxylamine probe, 1-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrrolidine, for *in vivo* measurements of reactive oxygen species: K. Saito, et al.; Free Radic. Biol. Med. **36**, 517 (2004)

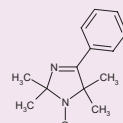


New Spin Probe

NEW PTMIO

[4-Phenyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl nitroxide]

ALX-430-145-M050	50 mg
ALX-430-145-M250	250 mg
ALX-430-145-G001	1 g



Biradical Spin Label

For Monitoring of Intracellular Redox States of Thiols

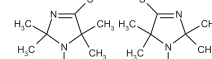
RSSR

[bis-(2,2,5,5-Tetramethyl-3-imidazoline-1-oxyl-4-yl)disulfide, biradical]

ALX-430-102-M010	10 mg
ALX-430-102-M025	25 mg
ALX-430-102-M050	50 mg

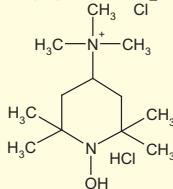
Cell permeable supersensitive spin label (detection limit varies from 10nM to 100nM) for quick detection of reduced thiols using ESR. Allows following the reactions of sulfhydryl groups with RSSR to form thiol spin label adducts, for the monitoring of intracellular redox states of glutathione and other thiols.

LIT: Quantitative determination of SH groups in low- and high-molecular-weight compounds by an electron spin resonance method: V.V. Khrantsov, et al.; Anal. Biochem. **182**, 58 (1989) • Quantitative determination of thiol groups in low and high molecular weight compounds by electron paramagnetic resonance: L.M. Weiner; Meth. Enzymol. **251**, 87 (1995) • Quantitative determination and reversible modification of thiols using imidazolidine biradical disulfide label: V.V. Khrantsov, et al.; J. Biochem. Biophys. Methods **35**, 115 (1997) • Use of imidazolidine nitroxides in studies of chemical reactions. ESR measurements of the concentration and reactivity of protons, thiols and nitric oxide: V.V. Khrantsov & B. Volodarsky; Spin labeling. The next Millennium. **145**, 109 (1998) • Unique *in vivo* applications of spin traps: L.J. Berliner, et al.; Free Radic. Biol. Med. **30**, 489 (2001) • *In vitro* and *in vivo* measurement of pH and thiols by EPR-based techniques: V.V. Khrantsov, et al.; Antioxid. Redox. Signal. **6**, 667 (2004)

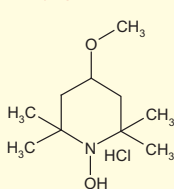


Chemical Structures

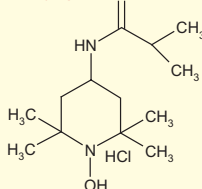
CAT1-H · HCl



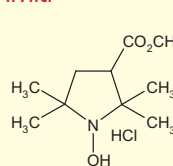
TM-H · HCl



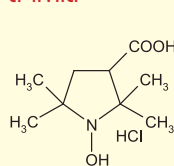
TMT-H · HCl



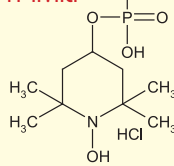
CM-H · HCl



CP-H · HCl



PP-H · HCl



Wide Range of Other Spin Traps & Spin Probes

CDMIO . K

[4-Carboxy-2,2-dimethyl-2H-imidazole-1-oxide . K]

ALX-430-089-M010 10 mg

ALX-430-089-M050 50 mg

Water soluble, non-cell permeable spin trap. Stable against reduction by vitamin C and thiols.

LIT: 2H-imidazole-N-oxides as spin traps: S.I. Dikalov, et al.; Bull. Russ. Acad. Sci. 41, 834 (1992) • Spin trapping of O₂⁻, C₂, and S-centered radicals and peroxynitrite by 2H-imidazole-1-oxides: S.I. Dikalov, et al.; BBRC 218, 616 (1996)

DMPIO

[2,2-Dimethyl-4-phenyl-2H-imidazole-1-oxide]

ALX-430-088-M010 10 mg

ALX-430-088-M050 50 mg

Widely used, cell permeable and highly sensitive spin trap. Alternative to PBN (Prod. No. ALX-430-082). More lipophilic than TMIO (Prod. No. ALX-430-073). Does not trap the superoxide radical.

LIT: Oxygen-centered spin adducts of 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 2H-imidazole-1-oxides: A.G. Krainev, et al.; J. Magn. Reson. Series B. 111, 272 (1996) • Spin trapping of O₂⁻, C₂, and S-centered radicals and peroxynitrite by 2H-imidazole-1-oxides: S.I. Dikalov, et al.; BBRC 218, 616 (1996)

MCPIO

[2-(2-Carboxyethyl)-2-methyl-4-phenyl-2H-imidazole-1-oxide]

ALX-430-083-M010 10 mg

ALX-430-083-M050 50 mg

Cell permeable, stable spin trap. Alternative to PBN (Prod. No. ALX-430-082).

LIT: 2H-imidazole-N-oxides as spin traps: S.I. Dikalov, et al.; Bull. Russ. Acad. Sci. 41, 834 (1992) • Spin trapping of O₂⁻, C₂, and S-centered radicals and peroxynitrite by 2H-imidazole-1-oxides: S.I. Dikalov, et al.; BBRC 218, 616 (1996)

PBON (high purity)

[α-(4-Pyridyl 1-oxide)-N-tert-butyl-nitron]

ALX-430-091-M500 500 mg

ALX-430-091-G001 1 g

Cell permeable hydrophilic spin trap for both *in vivo* and *in vitro* studies. Water soluble analog of PBN (Prod. No. ALX-430-082). Low paramagnetic impurities.

LIT: Two decades of spin trapping: E.G. Janzen & D.L. Hare; Adv. Radic. Chem. 1, 253 (1990) • In vitro and in vivo evidence for the formation of methyl radical from procarbazine: a spin-trapping study: L. Goria-Gatti, et al.; Carcinogenesis 13, 799 (1992) • Polyunsaturated fatty acids increase lipid radical formation induced by oxidant stress in endothelial cells: L.S. Alexander-North, et al.; J. Lipid Res. 35, 1773 (1994) • For a comprehensive bibliography please visit our website.

TEMPONE

[4-Oxo-2,2,6,6-tetramethylpiperidine-1-oxyl; 4-Oxo-TEMPO]

ALX-430-079-M250 250 mg

ALX-430-079-M500 500 mg

ALX-430-079-G001 1 g

Spin trap, useful for both *in vitro* and *in vivo* experiments.

LIT: Cytotoxicity of commonly used nitroxide radical spin probes: E.G. Ankel, et al.; Life Sci. 40, 495 (1987) • Biologically active metal-

independent superoxide dismutase mimics: J.B. Mitchell, et al.; Biochemistry 29, 2802 (1990) • Superoxide reaction with nitroxides: A. Samuni, et al.; Free Rad. Res. Commun. 9, 241 (1990) • Inhibition of oxygen-dependent radiation-induced damage by the nitroxide superoxide dismutase mimic, tempol: J.B. Mitchell, et al.; Arch. Biochem. Biophys. 289, 62 (1991) • Nitron spin traps and a nitroxide antioxidant inhibit a common pathway of thymocyte apoptosis: A.F. Slater, et al.; Biochem. J. 306, 771 (1995) • Cytotoxicity of spin trapping compounds: R.F. Haseloff, et al.; FEBS Lett. 418, 73 (1997) • For a comprehensive bibliography please visit our website.

TEMPONE-H . HCl

[1-Hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine . HCl]

ALX-430-071-M010 10 mg

ALX-430-071-M050 50 mg

ALX-430-071-M250 250 mg

Very effective, cell permeable and non-toxic spin trap for the detection of superoxide radical and peroxynitrite.

LIT: Quantification of superoxide radicals and peroxynitrite in vascular cells using oxidation of sterically hindered hydroxylamines and electron spin resonance: S. Dikalov, et al.; Nitric Oxide Biol. Chem. 1, 423 (1997) • Quantification of peroxynitrite, superoxide, and peroxyl radicals by a new spin trap hydroxylamine 1-Hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine: S. Dikalov, et al.; BBRC 230, 54 (1997) • For a comprehensive bibliography please visit our website.

TMIO

[2,2,4-Trimethyl-2H-imidazole-1-oxide]

ALX-430-073-M050 50 mg

ALX-430-073-M250 250 mg

ALX-430-073-G001 1 g

Selective, cell permeable and non-toxic spin trap for peroxynitrite and secondary O₂⁻, C₂⁻, S₂⁻, and N₂⁻ centered free radicals.

LIT: Spin trapping of O₂⁻, C₂⁻, and S₂⁻ centered radicals and peroxynitrite by 2H-imidazole-1-oxides: S.I. Dikalov, et al.; BBRC 218, 616 (1996) • Determination of rate constants of the reactions of thiols with superoxide radical by electron paramagnetic resonance: critical remarks on spectrophotometric approaches: S. Dikalov, et al.; Arch. Biochem. Biophys. 326, 207 (1996) • Spin trapping of superoxide radicals and peroxynitrite by 1-hydroxy-3-carboxy-pyrrolidine and 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine and the stability of corresponding nitroxyl radicals towards biological reductants: S. Dikalov, et al.; BBRC 231, 701 (1997) • For a comprehensive bibliography please visit our website.

TMPO

[3,3,5,5-Tetramethyl-pyrroline-N-oxide; M4PO]

ALX-430-084-M100 100 mg

ALX-430-084-M500 500 mg

Solid cell permeable spin trap.

LIT: Synthesis of spin traps specific for hydroxyl radical: G.M. Rosen & M.J. Turner, 3rd; J. Med. Chem. 31, 428 (1988) • The spin trapping of superoxide with M4PO (3,3,5,5-tetramethylpyrroline-N-oxide): G.R. Buettner & B.E. Britigan; Free Radic. Biol. Med. 8, 57 (1990) • Comparison of 2,5,5-trimethyl-1-pyrroline-N-oxide (M3PO) and 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (M4PO) with 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin traps: M. Nishi, et al.; Biochem. Int. 27, 651 (1992) • The effect of myoglobin on the stability of the hydroxyl radical adducts of 5,5 dimethyl-1-pyrroline-N-oxide (DMPO), 3,3,5,5 tetramethyl-1-pyrroline-N-oxide (TMPO) and 1-alpha-phenyl-tert-butyl nitron (PBN) in the presence of hydrogen: D. De Bono, et al.; Free Radic. Res. 20, 327 (1994) • For a comprehensive bibliography please visit our website.

Step
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Source™

Your Source for PBN!

Sold in Bulk Quantities!

PBN [N-tert-Butyl-α-phenylnitron]

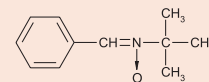
ALX-430-082-G001

1 g

For Bulk Quantities please inquire!

Cell permeable spin trap for both *in vivo* and *in vitro* studies.

LIT: For a comprehensive bibliography please visit our website.



Spin Trapping & Beer

The spin trapping agent PBN is used to investigate the influence of additives on the oxidative flavor stability of beer.



SELECTED LITERATURE REFERENCES

Detection of free radicals in beer oxidation: H. Kaneda, et al.; J. Food Sci. 53, 885 (1988)

The role of free radicals in beer oxidation: H. Kaneda, et al.; J. Am. Soc. Brew. Chem. 47, 49 (1989)

Improvement for oxidative flavor stability of beer - role of OH-radical in beer oxidation: M. Uchida & M. Ono; J. Am. Soc. Brew. Chem. 54, 198 (1996)

Electron spin resonance spin trapping identification of radicals formed during aerobic forced aging of beer: M.L. Andersen & L.H. Skibsted; J. Agric. Food Chem. 46, 1272 (1998)

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Electron paramagnetic resonance (EPR) profiling for potential flavor stability improvements in beer: R.T. Foster, et al.; MBAA TQ 38, 247 (2001)

The influence of additives on beer stability investigated by EPR spectroscopy: V. Brezova, et al.; Spectrochimica Acta (Part A) 58, 1279 (2002)

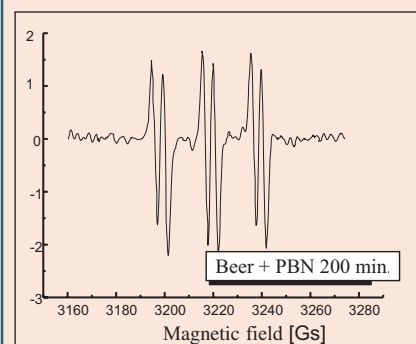
Determination of antioxidants in brewing: some aspects about the use of selected chemical and physical assays: A. Stephan, et al.; Eur. Brew. Conv. 31, 99 (2002)

Radicaloid-type oxidative decomposition of beer bittering agents revealed: K. Huvaere, et al.; Chemistry 9, 4693 (2003)

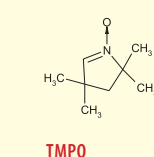
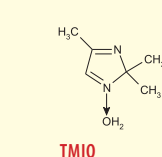
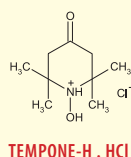
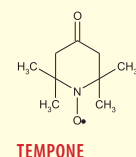
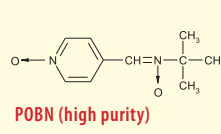
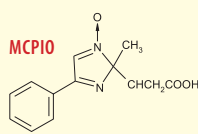
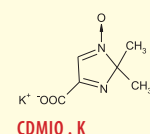
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Photooxidative degradation of beer bittering principles: a key step on the route to lightstruck flavor formation in beer: K. Huvaere, et al.; J. Agric. Food Chem. 53, 1489 (2005)

Antioxidant pool in beer and kinetics of EPR spin-trapping: N.M. Kocherginsky, et al.; J. Agric. Food Chem. 53, 6870 (2005)



Chemical Structures



Immuno-spin Trapping

a breakthrough for the sensitive detection of protein-derived and DNA radicals

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. 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)

Product Highlight

ALEXIS
BIOCHEMICALS

offers the widely published

anti-DMPO Polyclonal Antibody

ALX-210-530-R100

100 µl

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 histidyl 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. Kesler, 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)

Latest Insight

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, S.E. Mejiba, R.P. Mason; *Nat. Method.* **3**, 123 (2006)

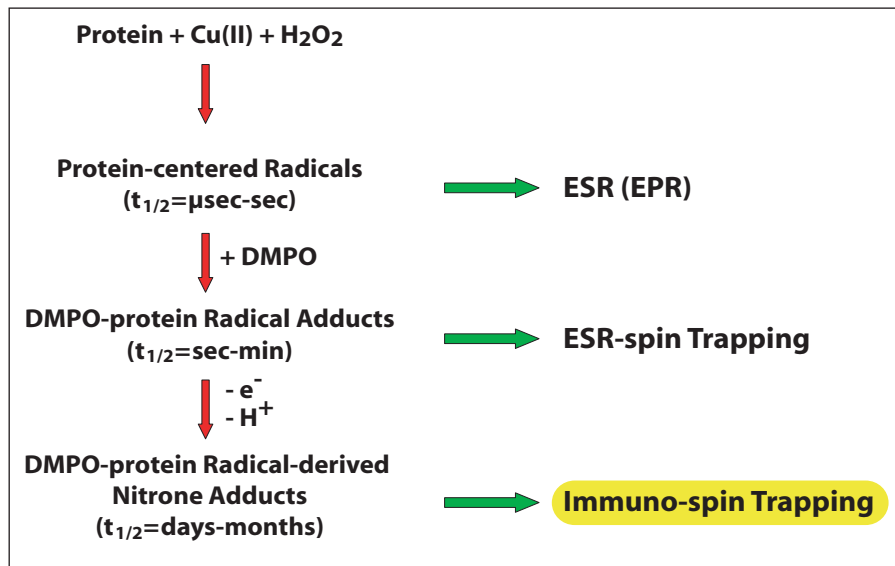


FIGURE: Schematic overview of approaches to detect protein radicals.

Nitric Oxide Spin-trapping Reagents

Diethyldithiocarbamic acid . Na . 3H₂O (high purity)

[DETC; Diethyldithiocarbamate]

ALX-400-003-G005 5 g
ALX-400-003-G025 25 g

Nitric oxide spin-trapping reagent. Thiol and iron chelator. Inhibits induction of macrophage nitric oxide synthase. Has been shown to be an inhibitor of the nuclear transcription factor κ B (NF- κ B).

LIT: On-line detection of nitric oxide formation in liquid aqueous phase by electron paramagnetic resonance spectroscopy: P. Mordvinov, et al.; *Anal. Biochem.* **199**, 142 (1991) • NO accounts completely for the oxygenated nitrogen species generated by enzymic L-arginine oxygenation: A. Mülisch, et al.; *Biochem. J.* **288**, 597 (1992) • Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells: R. Schreck, et al.; *J. Exp. Med.* **175**, 1181 (1992) • Diethyldithiocarbamate inhibits induction of macrophage NO synthase: A. Mülisch, et al.; *FEBS Lett.* **321**, 215 (1993) • The relationship between L-arginine-dependent nitric oxide synthesis, nitrite release and dinitrosyl-iron complex formation by activated macrophages: A. Vanin, et al.; *Biochim. Biophys. Acta* **1177**, 37 (1993) • Iron diethyldithiocarbamate as spin trap for nitric oxide detection: A.F. Vanin; *Methods Enzymol.* **301**, 269 (1999)

MGD . Na . H₂O

[N-(Dithiocarbamoyl)-N-Methyl-D-glucamine]

ALX-400-014-M050 50 mg
ALX-400-014-M250 250 mg

Together with FeSO₄ MGD is a useful component for the formation of the MGD₂-Fe²⁺ complex, which is an excellent nitric oxide (NO) spin-trapping reagent. The MGD₂-Fe²⁺ complex is quite unstable, especially in the presence of dissolved oxygen. Thus, the complex should be used immediately after being made. An excess (usually 5-fold excess), of MGD to Fe²⁺ is used for making the complex with FeSO₄ to give a more stable complex solution. Acidic conditions should be avoided because dithiocarbamate tends to decompose forming toxic carbon disulfide. It was reported that MGD and Fe(MGD)₂ do not exhibit toxicity up to 8mmol/kg and 0.3mmol/kg, respectively.

LIT: Sodium N-methyl-D-glucamine dithiocarbamate and cadmium intoxication: L.A. Shinobu, et al.; *Acta Pharmacol. Toxicol.* **54**, 189 (1984) • In vivo spin trapping of nitric oxide in mice: A. Komarov, et al.; *BBRC* **195**, 1191 (1993) • Spin trapping of nitric oxide produced in vivo in septic-shock mice: C.-S. Lei & A.M. Komarov; *FEBS Lett.* **345**, 120 (1994) • Spin trapping isotopically-labelled nitric oxide produced from [15N]-L-arginine and [17O]dioxigen by activated macrophages using a water soluble Fe(++)-dithiocarbamate spin trap: Y. Kotake, et al.; *Free Rad. Res.* **23**, 287 (1995) • Continuous monitoring of cellular nitric oxide generation by spin trapping with an iron-dithiocarbamate complex: Y. Kotake, et al.; *Biochim. Biophys. Acta* **1289**, 362 (1996) • Continuous and quantitative monitoring of rate of cellular nitric oxide generation: Y. Kotake; *Methods Enzymol.* **268**, 222 (1996) • Redox properties of iron-dithiocarbamates and their nitrosyl derivatives: implications for their use as traps of nitric oxide in biological systems: A.F. Vanin, et al.; *Biochim. Biophys. Acta* **1474**, 365 (2000) • EPR spectroscopy of common nitric oxide - spin trap complexes: S. Nedeianu & T. Pali; *Cell. Mol. Biol. Lett.* **7**, 142 (2002)

Trimethylammonio-PTIO

ALX-430-085-M010 10 mg
ALX-430-085-M050 50 mg

Water soluble nitric oxide (NO) spin trap that is non-cell permeable, allowing study of extracellular NO release.

LIT: Spin trapping of nitric oxide by nitronitroxides: measurement of the activity of NO synthase from rat cerebellum: Y.Y. Woldman, et al.; *BBRC* **202**, 195 (1994) • ESR study of free decomposition of N-bis(aryl)sulfonylhydroxylamines in organic solution: M.Y. Balakirev & V.V. Khramtsov; *J. Org. Chem.* **61**, 7263 (1996)

Selected Latest Review Articles

Electron spin resonance spin-trapping detection of superoxide generated by neuronal nitric oxide synthase: J. Vasquez-Vivar, et al.; *Methods Enzymol.* **301**, 169 (1999)

Detection and imaging of endogenously produced nitric oxide with electron paramagnetic resonance spectroscopy: S. Fujii & T. Yoshimura; *Antioxid. Redox Signal.* **2**, 879 (2000)

In vivo spin trapping of nitric oxide: L.J. Berliner & H. Fujii; *Antioxid. Redox Signal.* **6**, 649 (2004)

Simultaneous detection of NO and ROS by ESR in biological systems: Y. Cao, et al.; *Methods Enzymol.* **396**, 77 (2005)

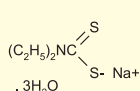
The ESR method to determine nitric oxide in plants: Y. Xu, et al.; *Methods Enzymol.* **396**, 84 (2005)

Application of electron spin resonance spin-trapping technique for evaluation of substrates and inhibitors of nitric oxide synthase: K. Saito & M. Kohno; *Anal. Biochem.* **349**, 16 (2006)

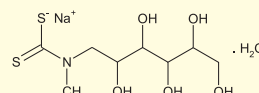
Inefficient spin trapping of superoxide in the presence of nitric-oxide: implications for studies on nitric-oxide synthase uncoupling: M. Pignitter, et al.; *Free Radic. Biol. Med.* **41**, 455 (2006)

Electron paramagnetic resonance (EPR) spin trapping of biological nitric oxide: A.L. Kleschyov, et al.; *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* (in press) (2006)

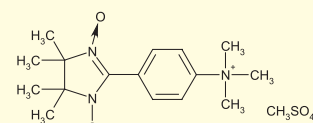
Chemical Structures



Diethyldithiocarbamic acid . Na . 3H₂O (high purity)



MGD . Na . H₂O



Trimethylammonio-PTIO

Antioxidant Spin Probe – A Key Standard Compound

TEMPOL

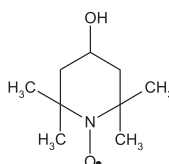
[4-Hydroxy-TEMPO; 4-Hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical]

ALX-430-081-M250 250 mg
ALX-430-081-M500 500 mg
ALX-430-081-G001 1 g

Free radical scavenger useful for both *in vivo* and *in vitro* experiments.

LIT: Measurement of intracellular oxygen concentration using the spin label TEMPOL: P.D. Morse, 2nd & H.M. Swartz; *Magn. Reson. Med.* **2**, 114 (1985) • Inhibition of oxygen-dependent radiation-induced damage by the nitroxide superoxide dismutase mimic, tempol: J.B. Mitchell, et al.; *Arch. Biochem. Biophys.* **289**, 62 (1991) • Tempol, a stable free radical, is a novel murine radiation protector: S.M. Hahn, et al.; *Cancer Res.* **52**, 1750 (1992) • Protective effect of 4-hydroxy-TEMPO, a low molecular weight superoxide dismutase mimic, on free radical toxicity in experimental pancreatitis: Z. Sledzinski, et al.; *Int. J. Pancreatol.* **18**, 153 (1995) • A novel antioxidant alleviates heat hyperalgesia in rats with an experimental painful peripheral neuropathy: M. Tal; *Neuroreport* **7**, 1382 (1996) • Stable nitroxide radicals protect lipid acyl chains from radiation damage: A.M. Samuni & Y. Barenholz; *Free Radic. Biol. Med.* **22**, 1165 (1997) • Tempol inhibits neutrophil and hydrogen peroxide-mediated DNA damage: S.M. Hahn, et al.; *Free Radic. Biol. Med.* **23**, 879 (1997) • Effects of the superoxide dismutase-mimic compound TEMPOL on oxidant stress-mediated endothelial dysfunction: A.I. Haj-Yehia, et al.; *Antioxid. Redox. Signal.* **1**, 221 (1999) • The nitroxide tempol induces oxidative stress, p21(WAF1/CIP1), and cell death in HL60 cells: M.B. Gariboldi, et al.; *Free Radic. Biol. Med.* **29**, 633 (2000) • Nitroxide TEMPOL impairs mitochondrial function and induces apoptosis in HL60 cells: E. Monti, et al.; *J. Cell. Biochem.* **82**, 271 (2001) • Spin trapping agents (Tempol and POBN) protect HepG2 cells overexpressing CYP2E1 against arachidonic acid toxicity:

M.J. Perez and A.I. Cederbaum; *Free Radic. Biol. Med.* **30**, 734 (2001) • Systemic arterial pressure response to two weeks of Tempol therapy in SHR: involvement of NO, the RAS, and oxidative stress: L. Yanes, et al.; *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288**, R903 (2005) • Tempol, one of nitroxides, is a novel ultraviolet-A1 radiation protector for human dermal fibroblasts: S.X. Yan, et al.; *J. Dermatol. Sci.* **37**, 137 (2005) • Cancer chemoprevention by the antioxidant tempol acts partially via the p53 tumor suppressor: L. Erker, et al.; *Hum. Mol. Genet.* **14**, 1699 (2005) • Antioxidant enzymes and effects of tempol on the development of hypertension induced by nitric oxide inhibition: J. Sainz, et al.; *Am. J. Hypertens.* **18**, 871 (2005) • Neuroprotective effects of TEMPOL in central and peripheral nervous system models of Parkinson's disease: Q. Liang, et al.; *Biochem. Pharmacol.* **70**, 1371 (2005) • The role of oxidant stress in angiotensin II-mediated contraction of human resistance arteries in the state of health and the presence of cardiovascular disease: M.B. Hussain, et al.; *Vascul. Pharmacol.* in print, (2006) • Acute effects of the superoxide dismutase mimetic tempol on split kidney function in two-kidney one-clip hypertensive rats: G.S. Guron, et al.; *J. Hypertens.* **24**, 387 (2006) • The effects of tempol, 3-aminobenzamide and nitric oxide synthase inhibitors on acoustic injury of the mouse cochlea: H. Murashita, et al.; *Hear. Res.* **214**, 1 (2006) • The nitroxide Tempol modulates anthracycline resistance in breast cancer cells: M.B. Gariboldi, et al.; *Free Radic. Biol. Med.* **40**, 1409 (2006)



Fluorinated Spin Probe

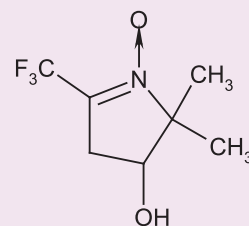
FDMPPO

[4-Hydroxy-5,5-dimethyl-2-trifluoromethylpyrroline-1-oxide]

ALX-430-135-M010 10 mg
ALX-430-135-M050 50 mg

A fluorinated spin trap which allows ¹⁹F-NMR detection of free radical reactions. Greatly improved signal-to-noise ratio when compared to the ³¹P-sensitivity of the phosphorus-containing spin trap DEPMPO (Prod. No. ALX-430-093).

LIT: NMR spin trapping: detection of free radical reactions with a new fluorinated DMPO analog: V.V. Khramtsov, et al.; *Free Radic. Biol. Med.* **30**, 1099 (2001)



NEW**pH-sensitive Spin Probes****DEDPI**

[4-(Dimethylamino)-2-ethyl-5,5-dimethyl-2-pyridine-4-yl-2,5-dihydro-1H-imidazol-1-oxyl]

ALX-430-120-M010 10 mg
ALX-430-120-M050 50 mg

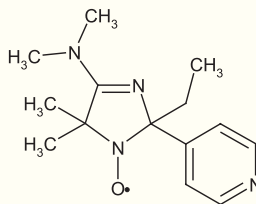
pH-sensitive nitroxide for *in vivo* studies. The presence of two ionizable groups in the side-chains extends the range of pH sensitivity. The relatively high solubility in water and broad range of pH-sensitivity makes the probe particularly suitable for pH monitoring in stomach using non-invasive low-field EPR techniques.

Latest Insight**Nitroxides with two pK values**

The group of I. A. Grigor'ev describes useful nitroxide spin probes for sensitive pH-monitoring within a broad range. This could be useful for many biophysical and biomedical applications, including pH-monitoring in the stomach.

FOR DETAILS SEE: *Nitroxides with two pK values – useful spin probes for pH monitoring within a broad range:* I. A. Kirilyuk, et al.; Org. Biomol. Chem. 3, 1269 (2005)

LIT: Grignard reagent addition to 5-alkylamino-4H-imidazole 3-oxides: synthesis of new pH-sensitive spin probes: T.G. Shevelev, et al.; Synthesis 2003, 871 • Synthesis of the tetraethyl substituted pH-sensitive nitroxides of imidazole series with enhanced stability towards reduction: I.A. Kirilyuk, et al.; Org. Biomol. Chem. 2, 1025 (2004) • In vitro and in vivo measurement of pH and thiols by EPR-based techniques: V.V. Khrantsov, et al.; Antioxid. Redox Signal. 6, 667 (2004) • Nitroxides with two pK values—useful spin probes for pH monitoring within a broad range: I.A. Kirilyuk, et al.; Org. Biomol. Chem. 3, 1269 (2005) • Real-time monitoring of drug-induced changes in the stomach acidity of living rats using improved pH-sensitive nitroxides and low-field EPR techniques: D.I. Potapenko, et al.; J. Magn. Reson. 182, 1 (2006)

**NEW****Efficient Cysteine-specific Spin Labelling Compound****MTSSL**

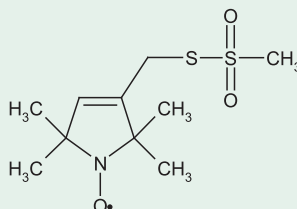
[(1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate]

ALX-430-134-M010 10 mg
ALX-430-134-M050 50 mg

Highly reactive thiol-specific spin label. Has been used to label cysteine residues in proteins (site-directed labelling, SDS-labelling). Allows protein structure and protein dynamics determination as well as the study of protein-protein and protein-oligonucleotide interactions.

LIT: A novel reversible thiol-specific spin label: papain active site labelling and inhibition: L.J. Berliner, et al.; Anal. Biochem. 119, 450 (1982) • Pressure-induced thermostabilization of glutamate dehydrogenase from the hyperthermophile Pyrococcus furiosus: M.M. Sun, et al.; Protein Sci. 8, 1056 (1999) • Methods for study of protein dynamics and protein-protein interaction in protein-ubiquitination by electron paramagnetic resonance spectroscopy: H.J. Steinhoff; Front. Biosci. 7, c97 (2002) • Protein structure determination using long-distance constraints from double-quantum coherence ESR: study of T4 lysozyme: P.P. Borbat, et al.; JACS 124, 5304 (2002) • Inter- and intra-molecular distances determined by EPR spectroscopy and site-directed spin labeling reveal protein-protein and protein-oligonucleotide interaction: H.J. Steinhoff; Biol. Chem. 385, 913 (2004) • Spontaneous refolding of the pore-forming Colicin A toxin upon membrane association as studied by X-band and W-band high-field electron paramagnetic resonance spectroscopy: A. Savitski, et al.; J. Phys. Chem. B 108, 9541

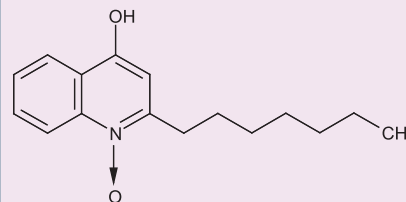
(2004) • Calcium structural transition of human cardiac troponin C in reconstituted muscle fibres as studied by site-directed spin labelling: M. Nakamura, et al.; J. Mol. Biol. 348, 127 (2005)

**Product Highlight****NEW****HQNO**

[2-n-Heptyl-4-hydroxyquinoline N-oxide]

ALX-430-130-M010 10 mg
ALX-430-130-M050 50 mg

Naturally occurring antagonist of dihydrostreptomycin. Potent inhibitor of the respiratory chain binding to the mitochondrial cytochrome b protein. Inhibits NADH oxidase (NADH) and Na⁺-dependent NADH-quinone reductase (NQR). Used in the investigation of the enzymatic pathways of elemental sulfur and thiosulfate disproportionation. Synthetic.



LIT: Inhibition of cytochrome system of heart muscle and of *Staphylococcus aureus* by 2-heptyl-4-hydroxyquinoline N-oxide, an antagonist of dihydrostreptomycin: J.W. Lightbown & E.L. Jackson; Biochem. J. 58, 15 (1954) • Structure of a naturally occurring antagonist of dihydrostreptomycin: J.W. Cornforth & A.T. James; Biochem. J. 63, 124 (1956) • Binding of HQNO to beef-heart sub-mitochondrial particles: G. Van Ark & J.A. Berden; Biochim. Biophys. Acta 459, 119 (1977) • Inhibitor studies of a new antibiotic, korormicin, 2-n-heptyl-4-hydroxyquinoline N-oxide and Ag⁺ toward the Na⁺-translocating NADH-quinone reductase from the marine *Vibrio alginolyticus*: Y. Nakayama, et al.; Biol. Pharm. Bull. 22, 1064 (1999) • FTIR spectroscopic evidence for the involvement of an acidic residue in quinone binding in cytochrome bd from *Escherichia coli*: J. Zhang, et al.; Biochemistry 41, 4612 (2002) • A stable isotope dilution assay for the quantification of the *Pseudomonas* quinolone signal in *Pseudomonas aeruginosa* cultures: F. Lepine, et al.; Biochim. Biophys. Acta 1622, 36 (2003) • Sulfite-oxido-reductase is involved in the oxidation of sulfite in *Desulfocapsa sulfoexigens* during disproportionation of thiosulfate and elemental sulfur: T.M. Frederiksen & K. Finster; Biodegradation 14, 189 (2003) • Enzymatic properties of the membrane-bound NADH oxidase system in the aerobic respiratory chain of *Bacillus cereus*: M.S. Kim & Y.J. Kim; J. Biochem. Mol. Biol. 37, 753 (2004) • Involvement of sulfide:quinone oxidoreductase in sulfur oxidation of an acidophilic iron-oxidizing bacterium, *Acidithiobacillus ferrooxidans* NASF-1: S. Wakai, et al.; Biosci. Biotechnol. Biochem. 68, 2519 (2004) • Physiological roles of three Na⁺/H⁺ antiporters in the halophilic bacterium *Vibrio parahaemolyticus*: T. Kuroda, et al.; Microbiol. Immunol. 49, 711 (2005) • Quinone reduction by *Rhodothermus marinus* succinate: menaquinone oxidoreductase is not stimulated by the membrane potential: A.S. Fernandes, et al.; BBR 330, 565 (2005) • Purification and characterization of succinate:menaquinone oxidoreductase from *Corynebacterium glutamicum*: T. Kurokawa and J. Sakamoto; Arch. Microbiol. 183, 317 (2005)

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