Product List — 19.2 Generating and Detecting Reactive Oxygen Species — continued

Cat #	Product Name	Unit Size
M-24571	merocyanine 540	25 mg
M-7913	*trans*-1-(2′-methoxyvinyl)pyrene	1 mg
M-23800	2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, hydrochloride (MCLA)	5 mg
M-7511	MitoTracker® Orange CM-H_2TMRos *special packaging*	20 x 50 µg
M-7513	MitoTracker® Red CM-H_2XRos *special packaging*	20 x 50 µg
M-6494	MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	1 g
N-6495	nitro blue tetrazolium chloride (NBT)	1 g
O-13291	OxyBURST® Green H_2HFF BSA *special packaging*	5 x 1 mg
P-1901	*cis*-parinaric acid *special packaging*	10 x 10 mg
P-6879	*N*-(1,10-phenanthrolin-5-yl)iodoacetamide	5 mg
P-800	B-phycoerythrin *4 mg/mL*	0.5 mL
P-801	R-phycoerythrin *4 mg/mL*	0.5 mL
R-14060	RedoxSensor™ Red CC-1 *special packaging*	10 x 50 µg
R-14000	rose bengal diacetate	5 mg
T-7539	2′,4′,5′,7′-tetrabromorhodamine 123 bromide	5 mg
X-6493	XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide)	100 mg

19.3 Probes for Nitric Oxide Research

Nitric oxide (NO), the molecule that makes the firefly glow,[1,2] plays a critical role as a molecular mediator of a variety of physiological processes, including blood-pressure regulation and neurotransmission.[3–8] In endothelial cells, as well as neurons and astrocytes, NO is synthesized from L-arginine in a reaction catalyzed by nitric-oxide synthase (NOS) (Figure 19.13). NO that diffuses into smooth muscle cells binds to the heme group of guanylate cyclase. Because free NO is a transient species with a half-life of about five seconds, many investigations of this gaseous molecule have relied largely on studies of NOS. Preparing NO solutions and detecting NO in experimental systems require special precautions to achieve reproducibility.[9] NO can also complex with superoxide to form the strong oxidant, peroxynitrite anion [10] ($ONOO^-$, Table 19.1), which may be a major cytotoxic agent produced during inflammation, sepsis and ischemia/reperfusion.[11] Activated macrophage and neutrophils produce nitric oxide and superoxide, and thus peroxynitrite anion, at similar rates.[12] NO generators are also reported to produce an accumulation of chelatable Zn^{2+} in hippocampal neuronal perikarya, as determined with some of our Zn^{2+} indicators [13] (Section 20.7, Table 20.6).

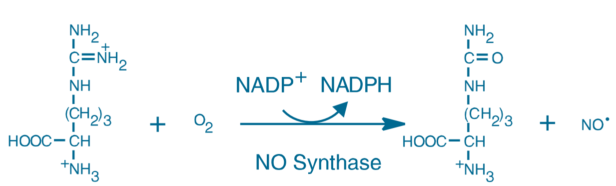

Figure 19.13 Nitric oxide synthase production of nitric oxide (NO) and L-citrulline from L-arginine and dioxygen (O_2).

Spontaneous Nitric Oxide Donors and Antagonist

DEANO and Spermine NONOate

The DEANO (diethylamine nitric oxide, D-7915) and spermine NONOate (S-7916) solids provide a means of preparing aqueous NO solutions.[14] When these solids are dissolved in buffer, cell culture medium or blood, they dissociate to form two molecules of NO and one molecule of the corresponding amine [15] (Figure 19.14). The delivery of NO can be easily controlled by preparing moderately basic solutions of these NONOates and then lowering the pH to initiate NO generation.

DEANO has a half-life of about two minutes in pH 7.4 phosphate buffer at 37°C, releasing two molecules of NO and one molecule of diethylamine.[14–17] DEANO therefore allows more sustained delivery of NO than is possible using NO-saturated aqueous solutions (Figure 19.15). The data in Figure 19.15 are from a study of cytochrome P-450 inhibition by NO in which DEANO was used in conjunction with fluorogenic microsomal dealkylase substrates [18] (R-441, R-352; Section 10.6). DEANO has also been used as a NO donor to stimulate ADP-ribosylation of various proteins.[19]

With a half-life of 39 minutes at 37°C in pH 7.4 buffer, spermine NONOate releases NO much more slowly than does DEANO, making it suitable for whole animal infusions and experiments with long incubations.[15] Also, spermine, the by-product of spermine NONOate dissociation, is less likely to be deleterious in living systems and may also demonstrate biological activity of its own.[20] Both DEANO and spermine NONOate are reported to inhibit *in vitro* proliferation of A375 melanoma cells.[21]

SNAP and SIN-1

Molecular Probes also offers the NO donors SNAP (*S*-nitroso-*N*-acetylpenicillamine, N-7892, N-7927) and SIN-1 (3-morpholinosydnonimine, hydrochloride; M-7891, M-7926), which spontaneously release NO (and superoxide in the case of SIN-1) under physiological conditions (Figure 19.14), thereby stimulating cyclic GMP production.[22–26] SNAP and SIN-1 have been shown to be potent vasodilators *in vivo* and *in vitro* and to inhibit smooth muscle cell mitogenesis and proliferation.[27–30] The relationship between NO generated from SNAP and SIN-1 and intracellular Ca^{2+} has been studied using fluorescent Ca^{2+} indicators [31–33] (Chapter 20). It has also been reported that NO released from SNAP stimulates Ca^{2+}-independent synaptic vesicle release,[33] which can be detected with FM 1-43 (T-3163, Section 16.1). We offer SNAP and SIN-1 in 25 mg vials (N-7892, M-7891) and, because of their potential instability in solution, as specially packaged sets of 20 vials, each containing 1 mg (N-7927, M-7926).

Carboxy PTIO: A Nitric Oxide Antagonist

Molecular Probes offers carboxy-PTIO (C-7912), a water-soluble and stable free radical molecule that reacts stoichiometrically with NO.[34–36] Carboxy-PTIO can be used *in vivo* to inhibit the physiological effects mediated by NO,[34,36,37] or *in vitro* to quantitate NO levels by ESR spectrometry.[38,39]

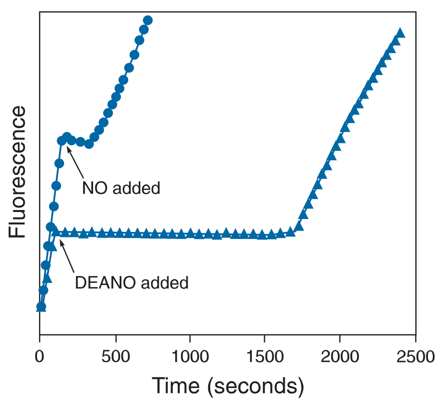

Figure 19.15 Inhibition of phenobarbital-induced rat hepatic microsomal dealkylase activity by 100 µM NO (●, added from a saturated aqueous stock solution) or 50 µM DEANO (▲, D-7915). Enzymatic activity was monitored using the fluorogenic substrate, resorufin benzyl ether (R-441). Reprinted with permission from Arch Biochem Biophys 300, 115 (1993).

SNAP: A Photoactivatable Nitric Oxide Donor

The potential spatial and temporal control of nitric oxide release made possible by photolysis of NO precursors makes this an attractive approach for generating NO in experimental systems. SNAP (*S*-nitroso-*N*-acetylpenicillamine, N-7892, N-7927) has been shown to release NO in response to light stimulation in both aqueous and isopropyl alcohol solutions.[40]

L-NMMA: A Nitric Oxide Synthase Inhibitor

Nitric oxide synthase catalyzes the NADPH- and O_2-dependent oxidation of L-arginine to NO and L-citrulline [41–43] (Figure 19.13). L-NMMA (N^G-methyl-L-arginine, M-7898) competitively inhibits NOS in a wide variety of cells, including neurons, endothelial cells and macrophages.[44,45]

Detecting Nitric Oxide, Nitrite and Nitrate

The nitric oxide (NO) radical is very unstable, and no equilibrium indicators for NO are known. However, NO is readily oxidized to the nitrosonium cation (NO^+), which is moderately stable in aqueous solutions but highly reactive with nucleophiles, or other nitrogen oxides. Under aerobic conditions, these reactive nitrogen oxides (Table 19.1) can be trapped by various amines, in particular by aromatic amines to form diazonium salts or aromatic 1,2-diamines to form benzotriazoles (Figure 19.16).

DAF-FM and DAF-FM Diacetate: The Preferred Indicators for Intracellular Nitric Oxide

Probably the most successful indicator for nitric oxide has been 4,5-diaminofluorescein diacetate (DAF-2 diacetate),[46,47] which was developed by Kojima and collaborators. As with other fluorescein diacetates (Section 15.2), DAF-2 diacetate is membrane permeant and is deacetylated by intracellular esterases to 4,5-diaminofluorescein (DAF-2). DAF-2, however, remains essentially nonfluorescent until it reacts with the nitrosonium cation (produced by spontaneous oxidation of nitric oxide) to form a fluorescent heterocycle, which becomes trapped in the cell's cytoplasm. DAF-2 has been used to identify individual nitric oxide–producing neurons in brain slices,[48,49] in mitochondria [50] and in living plant cells.[51] Simultaneous

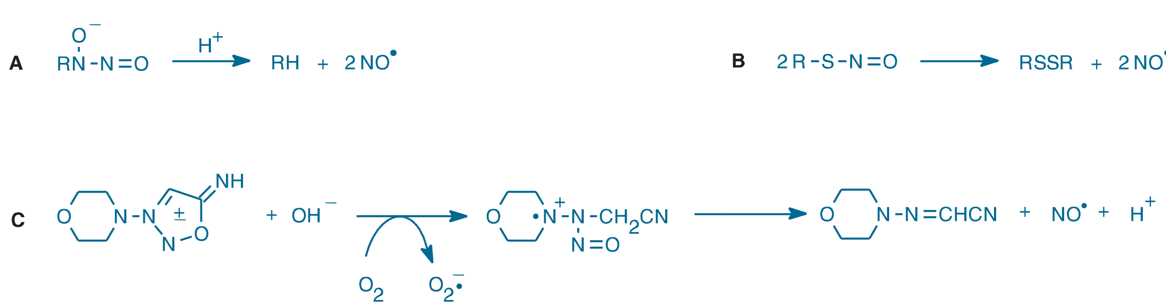

Figure 19.14 Mechanisms of spontaneous NO release by: A) DEANO (D-7915) or spermine NONOate (S-7916); B) SNAP (N-7892, N-7927); and C) SIN-1 (M-7891, M-7926).

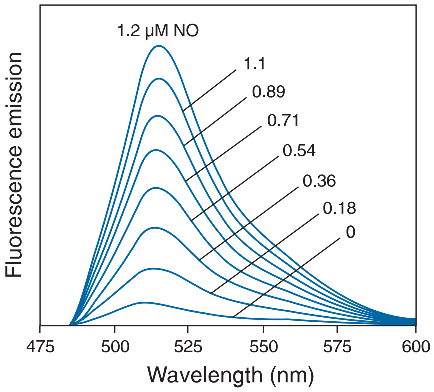

Figure 19.17 Fluorescence emission spectra of DAF-FM (D-23841, D-23842) in solutions containing zero to 1.2 μM nitric oxide (NO).

DAF-FM is currently the best probe available for detection of nitric oxide (NO) formation in live cells.

measurements of intracellular Ca^{2+} with fura-2 and nitric oxide production with DAF-2 have been reported.[52]

DAF-FM[52] (4-amino-5-methylamino-2′,7′-difluorofluorescein, D-23841) and its diacetate derivative[53] (DAF-FM diacetate, D-23842; D-23844) have significant utility for measuring nitric oxide production in living cells or solutions[54] (Figure 19.16, Figure 19.17). The fluorescence quantum yield of DAF-FM is reported to be 0.005 but increases about 160-fold to 0.81 after reacting with NO.[52] However, the DAF-FM reagent has some important advantages over DAF-2:

- Spectra of the NO adduct of DAF-FM are essentially independent of pH above pH 5.5[52]
- The NO adduct of DAF-FM is significantly more photostable than that of DAF-2[52]
- DAF-FM is a more sensitive reagent for NO than is DAF-2 (NO detection limit for DAF-FM ~3 nM[52] versus ~5 nM for DAF-2[46]

We anticipate that DAF-FM should give equal or superior performance to DAF-2 in most applications.

Griess Reagent Kit

Under physiological conditions, NO is readily oxidized to nitrite and nitrate or it is trapped by thiols as an *S*-nitroso adduct. The Griess reagent provides a simple and well characterized colorimetric assay for nitrites, and nitrates that have been reduced to nitrites, with a detection limit of about 100 nM.[55–57] Nitrites react with sulfanilic acid in acidic solution to form an intermediate diazonium salt that couples to *N*-(1-naphthyl)ethylenediamine to yield a purple azo derivative that can be monitored by absorbance at 548 nm (Figure 19.18). Our Griess Reagent Kit (G-7921) contains all of the reagents required for nitrite quantitation, including:

- *N*-(1-Naphthyl)ethylenediamine dihydrochloride
- Sulfanilic acid in 5% H_3PO_4
- A concentrated nitrite standard for generating calibration curves
- Detailed protocols for spectrophotometer and microplate reader assays

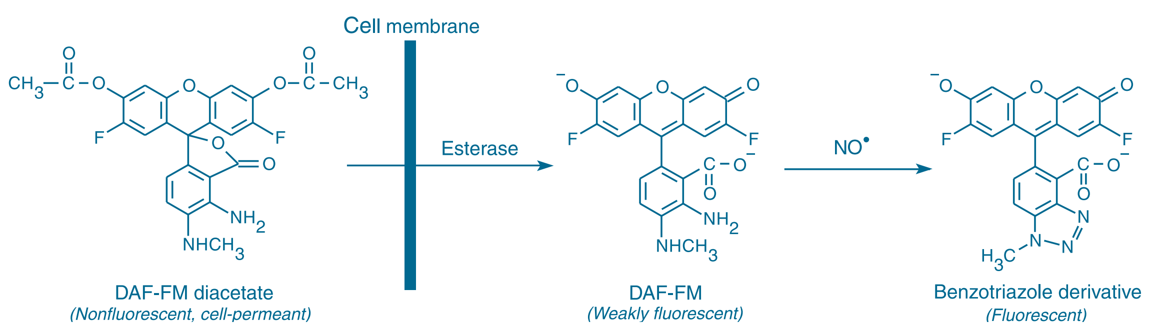

Figure 19.16 Reaction scheme for the detection of nitric oxide (NO) by DAF-FM (D-23841) and DAF-FM diacetate (D-23842, D-23844).

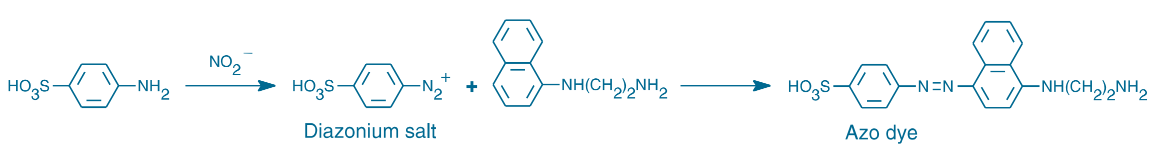

Figure 19.18 Principle of nitrite quantitation using the Griess Reagent Kit (G-7921). Formation of the azo dye is detected via its absorbance at 548 nm.

Both the *N*-(1-naphthyl)ethylenediamine dihydrochloride and the sulfanilic acid in 5% H_3PO_4 are provided in convenient dropper bottles for easy preparation of the Griess reagent. Sample pretreatment with nitrate reductase and glucose 6-phosphate dehydrogenase is reported to reduce nitrate without producing excess NADPH, which can interfere with the Griess reaction.[58] The Griess Reagent Kit can also be used to analyze NO that has been trapped as an *S*-nitroso derivative by a modificaition that uses mercuric chloride or copper (II) acetate to release the NO from its complex.[59,60]

2,3-Diaminonaphthalene

In a reaction similar to that of DAF-FM (Figure 19.16), 2,3-diaminonaphthalene (D-7918) reacts with the nitrosonium cation that forms spontaneously from NO to form the fluorescent product 1*H*-naphthotriazole.[55,61] Using 2,3-diaminonaphthalene, researchers have developed a rapid, quantitative fluorometric assay that can detect from 10 nM to 10 µM nitrite and is compatible with a 96-well microplate format.[62]

1,2-Diaminoanthraquinone

For directly detecting NO levels in vivo, we offer 1,2-diaminoanthraquinone (DAA, D-23840). This nitric oxide probe is reported to be nonfluorescent until it reacts with NO to produce a red-fluorescent precipitate. 1,2-Diaminoanthraquinone has been used to detect changes in NO levels in rat retinas after injury to the optic nerve.[63] This may make it possible to test the actions of NO in neurodegeneration, inflammation and other biological processes.

NBD Methylhydrazine

NBD methylhydrazine (*N*-methyl-4-hydrazino-7-nitrobenzofurazan, M-20490) is a unique reagent for the detection of nitrite. Reaction of NBD methylhydrazine with NO_2^- in the presence of mineral acids leads to formation of fluorescent products with excitation/emission maxima of ~468/537 nm. This reaction serves as the principle behind a selective fluorogenic method for the determination of NO_2^- (Figure 19.19). Although NBD methylhydrazine has been used to quantitate nitrite in water using a fluorescence microplate reader,[64] it does not seem to have been used yet to detect nitrite formed by spontaneous oxidation of NO.

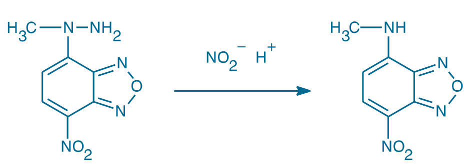

Figure 19.19 Reaction scheme illustrating the principle of nitrite detection by NBD methylhydrazine (M-20490).

Dichlorodihydrofluorescein Diacetate and Dihydrorhodamine 123

In addition to their extensive use for detecting other reactive oxygen species such as superoxide (Section 19.2), dichlorodihydrofluorescein diacetate (H_2DCFDA, D-399; Section 19.2) and dihydrorhodamine 123 (D-632, Section 19.2) have been reported to be useful for detecting peroxynitrite formation in both solution and in certain living cells.[10,65–69]

Anti-Nitrotyrosine Antibody

High levels of nitrotyrosine are associated with a large number of diseases, including multiple sclerosis, Alzheimer's and Parkinson's.[70] Increased levels of nitrotyrosine are also indicative of vascular and tissue injury from ischemia–reperfusion and inflammation.[71] At the protein level, tyrosine nitration can lead to loss or alteration of protein function, as demonstrated for the oncogenic protein p53[72] and the mitochondrial protein manganese superoxide dismutase.[73]

Several pathways for the nitration of tyrosine have been suggested. Nitric oxide (NO•) can interact rapidly with superoxide ($\cdot O_2^-$) to form peroxynitrite ($OONO^-$), a potent oxidant of aromatic, aliphatic and sulfhydryl residues.[10,73] Heme peroxidases, such as myeloperoxidase or eosinophil peroxidase, have been shown to utilize hydrogen peroxide (H_2O_2) to oxidize nitrite (NO_2^-) and catalyze tyrosine nitration.[74] Tryptophan residues can also be oxidized by peroxynitrite.[75]

Molecular Probes offers a high-activity rabbit polyclonal anti-nitrotyrosine antibody (A-21285) for detecting nitrotyrosine-containing proteins and peptides. This antibody is suitable for both immunohistochemical (Figure 7.67) and Western blotting applications (Figure 19.20) and should be useful for identifying nitrated proteins and determining the level of protein nitrosylation in tissues.

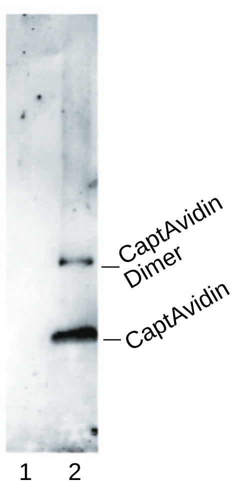

Figure 19.20 Specificity of our rabbit anti-nitrotyrosine antibody (A-21285) to nitrated proteins. Equal amounts of avidin (A-887, lane 1) and CaptAvidin biotin-binding protein (C-21385, lane 2) were run on an SDS-polyacrylamide gel (4–20%) and blotted onto a PVDF membrane. CaptAvidin biotin-binding protein, a derivative of avidin, has nitrated tyrosine residues in the biotin-binding site. Using the Pro-Q Western Blot Stain Kit #3 (P-21864), nitrated proteins were identified with the anti-nitrotyrosine antibody, in combination with an alkaline phosphatase conjugate of goat anti–rabbit IgG antibody (G-21079) and the red-fluorescent substrate, DDAO phosphate (D-6487).

References

1. Science 292, 2413 (2001); **2.** Science 292, 2486 (2001); **3.** Curr Biol 7, R376 (1997); **4.** J Med Chem 38, 4343 (1995); **5.** Annu Rev Biochem 63, 175 (1994); **6.** Cell 78, 919 (1994); **7.** Am J Physiol 262, G379 (1992); **8.** Science 257, 494 (1992); **9.** Methods Mol Biol 100, 215 (1998); **10.** Free Radic Biol Med 30, 463 (2001); **11.** Arch Biochem Biophys 298, 431 (1992); **12.** Methods Enzymol 233, 229 (1994); **13.** Brain Res 799, 118 (1998); **14.** Methods Enzymol 268, 281 (1996); **15.** J Med Chem 34, 3242 (1991); **16.** J Biol Chem 270, 17355 (1995); **17.** J Biol Chem 270, 655 (1995); **18.** Arch Biochem Biophys 300, 115 (1993); **19.** J Leukoc Biol 57, 152 (1995); **20.** (1989); **21.** Cancer Res 53, 564 (1993); **22.** Nature 364,

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626 (1993); **23.** Nature 375, 68 (1995); **24.** Brain Res 619, 344 (1993); **25.** FEBS Lett 315, 139 (1993); **26.** Thromb Res 70, 405 (1993); **27.** J Pharmacol Exp Ther 260, 286 (1992); **28.** Eur J Pharmacol 144, 379 (1987); **29.** J Pharmacol Exp Ther 248, 762 (1989); **30.** J Clin Invest 83, 1774 (1989); **31.** Am J Physiol 266, L9 (1994); **32.** Life Sci 54, 1449 (1994); **33.** Neuron 12, 1235 (1994); **34.** Biochemistry 34, 7177 (1995); **35.** Biochem Biophys Res Commun 202, 923 (1994); **36.** Biochemistry 32, 827 (1993); **37.** Infect Immun 61, 3552 (1993); **38.** Photochem Photobiol 61, 325 (1995); **39.** Life Sci 54, 185 (1994); **40.** Photochem Photobiol 67, 282 (1998); **41.** Biochem J 298, 249 (1994); **42.** Cell 78, 915 (1994); **43.** J Med Chem 37, 1899 (1994); **44.** FEBS Lett 294, 221 (1991); **45.** J Biol Chem 269, 32318 (1994); **46.** Anal Chem 70, 2446 (1998); **47.** Chem Pharm Bull (Tokyo) 46, 373 (1998); **48.** J Neurosci Methods 92, 101 (1999); **49.** Brain Res 852, 239 (2000); **50.** Biochem Biophys Res Commun 272, 129 (2000); **51.** Plant J 23, 817 (2000); **52.** Angew Chem Int Ed Engl 38, 3209 (1999); **53.** Molecular Probes has an agreement with Daiichi Pure Chemicals Co., Ltd., to manufacture and sell DAF-FM and DAF-FM diacetate. These two products are available in Japan only through Daiichi Pure Chemicals Co. and their authorized distributors; **54.** Anal Biochem 287, 203 (2000); **55.** Luminescence 14, 283 (1999); **56.** FASEB J 7, 349 (1993); **57.** Anal Biochem 126, 131 (1982); **58.** Anal Biochem 224, 502 (1995); **59.** Anal Biochem 238, 150 (1996); **60.** Chem Biol 3, 655 (1996); **61.** Methods Enzymol 268, 105 (1996); **62.** Anal Biochem 214, 11 (1993); **63.** Neuroreport 9, 4051 (1998); **64.** Anal Chem 71, 3003 (1999); **65.** J Biol Chem 273, 12716 (1998); **66.** FEBS Lett 416, 175 (1997); **67.** Free Radic Res 27, 245 (1997); **68.** Clin Hemorheol 16, 685 (1996); **69.** Free Radic Biol Med 20, 373 (1996); **70.** Methods Enzymol 301, 373 (1999); **71.** Free Radic Res 33, 771 (2000); **72.** Biochem Biophys Res Commun 267, 609 (2000); **73.** Proc Natl Acad Sci U S A 93, 11853 (1996); **74.** Biochem Biophys Res Commun 285, 273 (2001); **75.** Biochem Biophys Res Commun 234, 82 (1997).

Data Table — 19.3 Probes for Nitric Oxide Research

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
C-7912	315.39	FF,D	H_2O	367	9,300	none	MeOH	
D-7915	155.13	FF,DD,A	H_2O, DMSO	248	8,000	none	pH 12	1
D-7918	158.20	L	DMSO, MeOH	340	5,100	377	MeOH	2
D-23840	336.32	F,D,L	DMSO	521	6,000	none	MeOH	3
D-23841	412.35	F,D,L	DMSO	487	84,000	see Notes	pH 8	4
D-23842	496.42	F,D,L	DMSO	<300		none		5
D-23844	496.42	F,D,L	DMSO	<300		none		5
M-7891	206.63	FF,D,LL	DMSO, H_2O	291	11,000	none	pH 7	6
M-7898	248.28	D	H_2O	<300		none		
M-7926	206.63	FF,D,LL	DMSO, H_2O	291	11,000	none	pH 7	6
M-20490	209.16	F,L	MeCN	487	24,000	none	MeOH	7
N-7892	220.24	FF,D,LL	DMSO, H_2O	342	700	none	MeOH	6
N-7927	220.24	FF,D,LL	DMSO, H_2O	342	700	none	MeOH	6
S-7916	262.35	FF,DD,A	H_2O,DMSO	248	6,700	none	pH 12	1

For definitions of the contents of this data table, see "How to Use This Book" on page viii.

Notes

1. Releases nitric oxide upon acid-catalyzed dissociation in solution. Stable in alkaline solutions (Methods Enzymol 268, 281 (1996)).
2. Fluorescence of D-7918 is weak. Reaction with nitrite yields highly fluorescent 1*H*-naphthotriazole (Abs = 365 nm, Em = 415 nm in H_2O (pH 12)) (Methods Enzymol 268, 105 (1996)).
3. 1,2-Diaminoanthraquinone reacts with nitrite or nitric oxide to produce 1*H*-anthratriazole-6,11-dione, which forms a red-fluorescent (Em >580 nm) precipitate in water (Neuroreport 9, 4051 (1998)).
4. DAF-FM fluorescence is very weak. Reaction with nitric oxide generates a highly fluorescent benzotriazole deriviative with Abs = 495 nm (EC = 73,000 $cm^{-1}M^{-1}$), Em = 515 nm in pH 7.4 buffer (Angew Chem Int Ed Engl 38, 3209 (1999)).
5. Acetate hydrolysis and subsequent reaction with nitric oxide generate a highly fluorescent benzotriazole deriviative with Abs = 495 nm (EC = 73,000 $cm^{-1}M^{-1}$), Em = 515 nm in pH 7.4 buffer (Angew Chem Int Ed Engl 38, 3209 (1999)).
6. Spontaneously decomposes in solution.
7. NBD methylhydrazine reacts with nitrite in the presence of strong acid to form fluorescent N-methyl-4-amino-7-nitrobenzofurazan, Abs = 470 nm, Em = 547 nm in aqueous buffer (pH 5.8) (Anal Chem 71, 3003 (1999)).

Product List — 19.3 Probes for Nitric Oxide Research

Cat #	Product Name	Unit Size
A-21285	anti-nitrotyrosine, rabbit IgG fraction *1 mg/mL*	0.5 mL
C-7912	2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, potassium salt (carboxy-PTIO)	25 mg
D-23841	DAF-FM (4-amino-5-methylamino-2′,7′-difluorofluorescein)	1 mg
D-23842	DAF-FM diacetate (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate)	1 mg
D-23844	DAF-FM diacetate (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate) *special packaging*	10 x 50 µg
D-23840	1,2-diaminoanthraquinone sulfate (DAA) *high purity*	5 mg
D-7918	2,3-diaminonaphthalene	100 mg
D-7915	diethylamine nitric oxide, sodium salt (DEANO)	25 mg
G-7921	Griess Reagent Kit *for nitrite quantitation*	1 kit
M-7898	N^G-methyl-L-arginine, acetate salt (L-NMMA)	25 mg
M-20490	*N*-methyl-4-hydrazino-7-nitrobenzofurazan (NBD methylhydrazine)	25 mg
M-7891	3-morpholinosydnonimine, hydrochloride (SIN-1)	25 mg
M-7926	3-morpholinosydnonimine, hydrochloride (SIN-1) *special packaging*	20 x 1 mg
N-7892	*S*-nitroso-*N*-acetylpenicillamine (SNAP)	25 mg
N-7927	*S*-nitroso-*N*-acetylpenicillamine (SNAP) *special packaging*	20 x 1 mg
S-7916	spermine NONOate	10 mg