Potential-Sensitive ANEP Dyes

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
ANEP dye	1 mg or 5 mg lyophilized solid	• Room temperature • Desiccate • Protect from light	When stored as directed, product is stable for 6 months.
Approximate fluorescence excitation and emission, in nm: Absorption and fluorescence spectra of the ANEP dyes are highly dependent on their environment. Full spectra can be found at our website, probes.invitrogen.com.			

Introduction

The ANEP (aminonaphthylethenylpyridinium) dyes developed by Leslie Loew and colleagues [1,2] are the most consistently sensitive probes for detection of submillisecond membrane potential changes. Di-4-ANEPPS (D1199) has a fairly uniform 10% per 100 mV change in fluorescence intensity in a variety of tissue, cell and model membrane systems.[3] ANEP dyes undergo changes in their electronic structure, and consequently their fluorescence spectra, in response to changes in the surrounding electric field.[2] This optical response is sufficiently fast to detect transient potential changes in excitable cells, including single neurons, cardiac cells and intact tissue preparations. Furthermore, these dyes display a potential-dependent shift in their excitation spectra, thus permitting the quantitation of membrane potential using excitation ratio measurements.[4] Structural variations among the ANEP dyes confer suitability for specialized applications (Figure 1, Table 2).

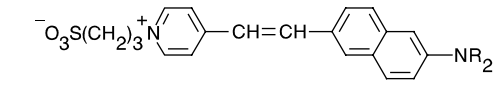
Guidelines for Use

Materials Required but Not Provided

- Solvent such as ethanol, DMSO, or DMF

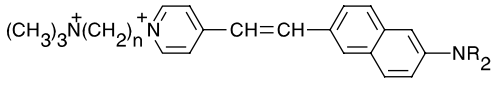
Preparing ANEP Dye Stock Solutions

Generally suitable stock solutions can be made in ethanol, DMSO, or DMF at 1 mg/mL (approximately 2 mM); store stock solutions protected from light at 2–6°C.



D1199 R = —$(CH_2)_3CH_3$

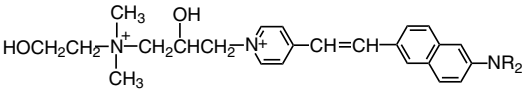
D3167 R = —$(CH_2)_7CH_3$



D6923 n = 2 R = —CH_2CH_3

D6925 n = 3 R = —$(CH_2)_7CH_3$

D6927 n = 3 R = —$(CH_2)_{11}CH_3$



D36801 R = –$(CH_2)_2CH_3$

D36802 R = –$(CH_2)_3CH_3$

Figure 1. Structures of ANEP dyes.

Spectral Characteristics

The spectral characteristics of ANEP dyes are all generally similar. Their maximum molar extinction coefficients for absorption in methanol are about 36,000 $cm^{-1}M^{-1}$ at about 498 nm (for the zwitterionic ANEPPS dyes) or 517 nm (for the cationic ANEPEQ and ANEPPQ dyes). Absorption and fluorescence spectra of the ANEP dyes are highly dependent on their environment.[1,9] The dyes are essentially nonfluorescent in water and become quite strongly fluorescent upon binding to membranes. The absorption and fluorescence emission maxima of di-8-ANEPPS bound to model phospholipid membranes are about 465 nm and 635 nm, respectively (full spectra can be found at our website, probes.invitrogen.com). Fluorescence is insensitive to pH and photostability is generally high.

Table 2. Functional characteristics of ANEP dyes.

Cat #	Name	Characteristics	Ref *
D1199	di-4-ANEPPS	Most consistent potentiometric response in different cell and tissue types	3
D3167	di-8-ANEPPS	Less susceptible to internalization than di-4-ANEPPS, permitting extended observation	5
D6923	di-2-ANEPEQ †	Highly water-soluble ANEP dye for microinjection	6
D6925	di-8-ANEPPQ	Most sensitive potentiometric response in retrogradely labeled neurons	7,8
D6927	di-12-ANEPPQ	Similar to di-8-ANEPPQ; useful for retrograde labeling of neurons	7,8
D36801	di-3-ANEPPDHQ	Exhibits very low internalization and good signal:noise ratio; useful for neural network analysis	12
D36802	di-4-ANEPPDHQ	Exhibits very low internalization and good signal:noise ratio; useful for neural network analysis and the visualization of cholesterol-enriched lipid domains	12, 13, 14

* Numbers refer to the main reference list below. † Also known as JPW 1114.

Loading Cells with ANEP Dyes

1. Bulk Loading. Cells attached to coverslips are typically labeled by direct addition of a small amount of dye stock solution in DMSO to the cell incubation medium to produce a loading concentration ranging from 0.2–2 µM. Higher dye concentrations (10–50 µM) are used for loading perfused tissue samples.[10,11] Pluronic® F-127 (0.05%) is often included in the loading solution to aid the solubilization of the dye (this is particularly necessary with the more lipophilic ANEP dyes such as di-8-ANEPPS). Molecular Probes offers Pluronic F-127 in three forms: 1 mL of a 20% (w/v) solution in DMSO (P3000), 30 mL of a sterile 10% (w/v) solution in water (P6866) and 2 g solid (P6867). Low incubation temperatures (4–20°C) inhibit internalization of the dye. After incubation for 10–20 minutes in the presence of the dye, cells are washed up to three times in dye-free medium.

2. Retrograde Labeling. Specific groups of neurons can be labeled by localized pressure injection of a concentrated dye solution (10–20 mg/mL in DMSO or ethanol) into neuronal tissue. Retrograde labeling of cell bodies with di-8-ANEPPQ (D6925) in chicken spinal cord was found to occur within 24 hours.[7]

3. Microinjection. Microelectrode tips filled with a 3 mg/mL (5.5 mM) aqueous solution of di-2-ANEPEQ (D6923) can be used for pressure microinjection into neurons.[6] The dye solution should be filtered immediately before filling to remove microscopic particles that may cause blockage of the electrode tip.

Fluorescence Measurements

Fluorescence ratio imaging measurements are typically performed by recording fluorescence intensities excited at about 450 and 510 nm, detecting emission at >570 nm.[3,4] The ratio of these intensities (F_{450}/F_{510}) decreases upon membrane hyperpolarization. Potential-dependent fluorescence emission ratio measurements (ratio of emission intensities at 560 nm and 620 nm, excitation at 475 nm) have also been reported using di-8-ANEPPS.[11] Calibration of the potentiometric response can be accomplished using the ionophore valinomycin (V1644) and varying membrane potential with the addition of different potassium concentrations to the extracellular medium.[3,4]

References

1. Biochemistry 24, 5749 (1985); **2.** Pure Appl Chem 68, 1405 (1996); **3.** J Membr Biol 130, 1 (1992); **4.** Biochemistry 28, 4536 (1989); **5.** Neuron 9, 393 (1992); **6.** J Neurosci 15, 1392 (1995); **7.** J Neurosci Methods 70, 111 (1996); **8.** J Neurosci Methods 70, 121 (1996); **9.** Biochim Biophys Acta 1323, 223 (1997); **10.** J Neurosci 16, 3456 (1996); **11.** Am J Physiol 270, H2216 (1996); **12.** J Neurosci Methods 134, 179 (2004); **13.** Biophys J 89, L04 (2005); **14.** J Exp Biol 208, 2981 (2005).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
D6923	di-2-ANEPEQ (JPW 1114)	5 mg
D6925	di-8-ANEPPQ	5 mg
D6927	di-12-ANEPPQ	5 mg
D1199	di-4-ANEPPS	5 mg
D3167	di-8-ANEPPS	5 mg
D36801	di-3-ANEPPDHQ	1 mg
D36802	di-4-ANEPPDHQ	1 mg
P6867	Pluronic® F-127 *low UV absorbance*	2 g
P3000	Pluronic® F-127 *20% solution in DMSO*	1 mL
P6866	Pluronic® F-127 *sterile 10% solution in water*	30 mL
V1644	valinomycin	25 mg

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License
For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300. Fax: (541) 335-0504.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.