

## Potential-Sensitive ANEP Dyes

**Table 1.** Contents and storage information.

Material	Amount	Storage	Stability
ANEP dye	1 mg or 5 mg lyophilized solid	<ul style="list-style-type: none"> <li>• Room temperature</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	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](http://probes.invitrogen.com).

### Introduction

The ANEP (aminonaphthylethenylpyridinium) dyes developed by Leslie Loew and colleagues<sup>1,2</sup> 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.<sup>3</sup> ANEP dyes undergo changes in their electronic structure, and consequently their fluorescence spectra, in response to changes in the surrounding electric field.<sup>2</sup> 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.<sup>4</sup> Structural variations among the ANEP dyes confer suitability for specialized applications (Figure 1, Table 2).

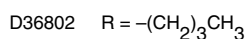
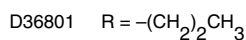
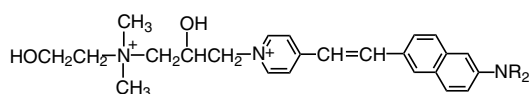
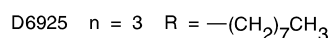
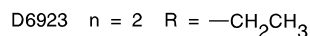
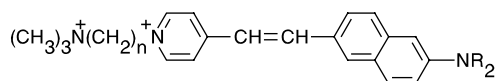
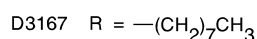
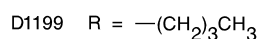
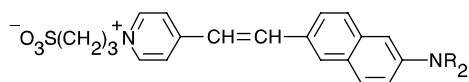
### Guidelines for Use

**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.



**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  $36,000 \text{ cm}^{-1}\text{M}^{-1}$  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.<sup>1,9</sup> 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  $\mu\text{M}$ . Higher dye concentrations (10–50  $\mu\text{M}$ ) are used for loading perfused tissue samples.<sup>10,11</sup> 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.<sup>7</sup>

**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.<sup>6</sup> 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.<sup>3,4</sup> 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.<sup>11</sup> 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.<sup>3,4</sup>

## References

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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

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

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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

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