

Cat #	Product Name	Unit Size
F-1176	fluorescein α -bungarotoxin (α -bungarotoxin, fluorescein conjugate)	500 μ g
G-7055	L-glutamic acid, γ -(α -carboxy-2-nitrobenzyl) ester, trifluoroacetic acid salt (γ -(CNB-caged) L-glutamic acid)	5 mg
M-7114	N-methyl-D-aspartic acid, β -(α -carboxy-2-nitrobenzyl) ester, trifluoroacetic acid salt (β -(CNB-caged) NMDA)	1 mg
M-23400	muscimol, BODIPY [®] TMR-X conjugate	1 mg
N-1384	naloxone fluorescein	5 mg
N-1385	naltrexone fluorescein	5 mg
N-13437	neuromedin C, Alexa Fluor [®] 488 conjugate	25 μ g
S-13426	substance P, Alexa Fluor [®] 488 conjugate	25 μ g
S-13425	substance P, BODIPY [®] FL conjugate	25 μ g
S-13424	substance P, fluorescein conjugate	25 μ g
S-13427	substance P, Oregon Green [®] 488 conjugate	25 μ g
S-13428	substance P, tetramethylrhodamine conjugate	25 μ g
T-1175	tetramethylrhodamine α -bungarotoxin (α -bungarotoxin, tetramethylrhodamine conjugate)	500 μ g

16.3 Probes for Ion Channels and Carriers

This section describes a variety of probes for Ca^{2+} , Na^+ , K^+ and Cl^- ion channels and carriers. Chapter 20, Chapter 21 and Chapter 22 contain our extensive selection of indicators for these physiologically important ions, providing a means of correlating ion channel activation with subsequent changes in intracellular ion concentration. Ion flux also affects the cell's membrane potential, which can be measured with the probes described in Chapter 23.

Probes for Ca^{2+} Channels and Carriers

In both excitable and nonexcitable cells, intracellular Ca^{2+} levels modulate a multitude of vital cellular processes — including gene expression, cell viability, cell proliferation, cell motility and cell shape and volume regulation — thereby playing a key role in regulating cell responses to external activating agents. These dynamic changes in intracellular Ca^{2+} levels are regulated by ligand-gated and G-protein-coupled ion channels in the plasma membrane, as well as by mobilization of Ca^{2+} from intracellular stores. One of the best-studied examples of Ca^{2+} -dependent signal transduction is the depolarization of excitable cells, such as those of neuronal, cardiac, skeletal and smooth muscle tissue, which is mediated by inward Ca^{2+} and Na^+ currents. The Ca^{2+} current is attributed to the movement of ions through N-, L-, P- and T-type Ca^{2+} channels, which are defined both pharmacologically and by their biophysical properties, including conductance and voltage sensitivity. Molecular Probes offers fluorescent analogs of dihydropyridine and verapamil as ligands for L-type Ca^{2+} channels. In addition, we offer unlabeled and fluorescent derivatives of ryanodine, a powerful modulator of the intracellular Ca^{2+} channels found in the sarcoplasmic reticulum and other subcellular organelles.

Fluorescent Dihydropyridines for L-Type Ca^{2+} Channels

The L-type Ca^{2+} channel is readily blocked by the binding of dihydropyridine to the channel's pore-forming α_1 -subunit. To facilitate the study of channel number and distribution in single cells, Molecular Probes has developed fluorescent dihydropyridine derivatives. The high-affinity (–)-enantiomer of dihydropyridine is

available labeled with either the green-fluorescent DM-BODIPY (D-7443, Figure 16.37) or the orange-fluorescent ST-BODIPY (S-7445) fluorophore. Knaus and colleagues have shown that these BODIPY dihydropyridines bind to L-type Ca^{2+} channels with high affinity and inhibit the Ca^{2+} influx in GH_3 cells.¹ DM-BODIPY dihydropyridine has been employed to investigate the molecular mechanism for dihydropyridine binding to L-type channels. Upon binding to the α_1 -subunit, this ligand is reported to exhibit an increase in fluorescence quantum yield, as well as fluorescence resonance energy transfer (FRET, see Section 1.3) between its fluorophore and one or more of the channel's tryptophan residues.² For neuronal L-type Ca^{2+} channels, the (–)-enantiomers of the DM-BODIPY dihydropyridine and ST-BODIPY derivatives each exhibit a K_i of 0.9 nM. Their affinities for skeletal muscle L-type Ca^{2+} channels are somewhat lower. Although DM-BODIPY dihydropyridine exhibits a more intense fluorescence, the particularly high degree of stereoselectivity retained by the ST-BODIPY derivatives has proven useful for the *in vivo* visualization of L-type Ca^{2+} channels.

The spatial distribution and density of L-type Ca^{2+} channels in cultured olfactory neurons were determined by confocal laser-scanning microscopy using the ratio of the site-selective fluorescent staining produced by the (–)-enantiomer of DM-BODIPY dihydropyridine (D-7443, Figure 16.37) to the uniform fluorescent membrane staining by RH 414³ (T-1111, Section 14.4). In this study, RH 414 staining served to control for optical artifacts and

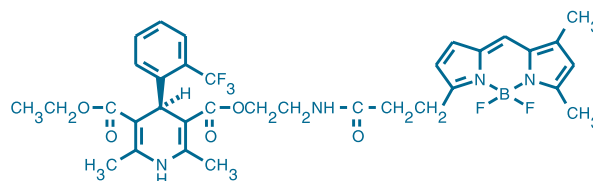


Figure 16.37 D-7443 DM-BODIPY (–)-dihydropyridine.



Figure 16.38 Cultured olfactory bulb neuron stained with RH 414 (T-1111) and DM-BODIPY(-) dihydropyridine (D-7443) (J Neurosci Methods 59, 183 (1995)). Top: image at >580 nm. Middle: image at 510–580 nm. Bottom: ratio of the middle image divided by the top image. Images were acquired with a Leica confocal laser-scanning microscope. Image contributed by D. Schild, H. Geiling and J. Bischofberger, Physiologisches Institut, Universität Göttingen.

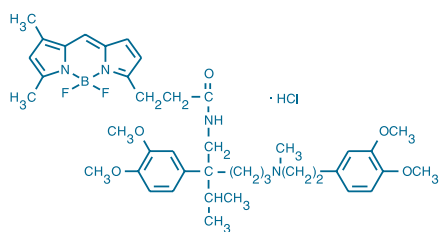


Figure 16.39 B-7431 BODIPY FL verapamil, hydrochloride.

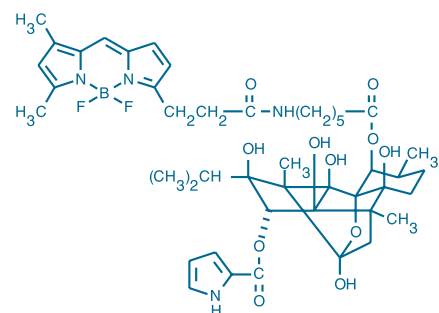


Figure 16.40 B-7505 BODIPY FL-X ryanodine.

differences in membrane surface area (Figure 16.38). The DM-BODIPY dihydropyridine and ST-BODIPY dihydropyridine have also been used to label dihydropyridine receptors in *Fucus* zygotes and embryos^{4,5} and to demonstrate the presence of L-type Ca^{2+} channels in peripheral blood-derived human dendritic cells⁶ and smooth muscle cells.⁷

BODIPY FL Verapamil

Like dihydropyridine, phenylalkylamines also bind to the α_1 -subunit of L-type Ca^{2+} channels and block Ca^{2+} transport. Molecular Probes offers a green-fluorescent BODIPY FL derivative (B-7431, Figure 16.39) of verapamil, a phenylalkylamine known to inhibit P-glycoprotein-mediated drug efflux. Although we do not yet know the effect of BODIPY FL verapamil on L-type Ca^{2+} channels, this fluorescent phenylalkylamine should be useful for investigating P-glycoprotein-mediated multidrug resistance.

The 170,000-dalton P-glycoprotein is typically overexpressed in tumor cells that have acquired resistance to a variety of anticancer drugs (Section 15.6). P-glycoprotein is thought to mediate the ATP-dependent efflux or sequestration of structurally unrelated molecules, including actinomycin D, anthracyclines, colchicine, epipodophyllotoxins and vinblastine. Verapamil appears to inhibit drug efflux by acting as a substrate of P-glycoprotein, thereby overwhelming the transporter's capacity to expel the drugs. BODIPY FL verapamil also appears to serve as a substrate for P-glycoprotein. This fluorescent verapamil derivative preferentially accumulates in the lysosomes of normal, drug-sensitive NIH 3T3 cells but is rapidly transported out of multidrug-resistant cells, as revealed by fluorescence microscopy.⁸

Ryanodine Probes for Intracellular Ca^{2+} Channels

Ryanodine is a plant alkaloid that mobilizes Ca^{2+} from intracellular stores by activating a class of Ins 1,4,5- P_3 -insensitive Ca^{2+} channels.⁹ It alters the function of the Ca^{2+} channel in a complex manner: submicromolar concentrations lock the channel in a long-lived open state, whereas micromolar or greater concentrations inhibit Ca^{2+} release.^{10,11} Ryanodine has been used to modulate the Ca^{2+} concentration in sea urchin eggs¹² and in parotid acinar cells.¹³ In developing skeletal muscle, ryanodine receptors localize in discrete regions of the T tubules, binding at the junctional complex between the T tubules and the sarcoplasmic reticulum.¹⁴

Unlabeled “ryanodine” from most other commercial sources is typically a mixture of ryanodine and dehydroryanodine.^{15,16} However, Molecular Probes offers an HPLC-purified grade of ryanodine (R-7478) that is $>95\%$ pure. In addition to unlabeled ryanodine, we have prepared a monosubstituted BODIPY FL-X derivative (B-7505) and BODIPY TR-X derivative (B-13802), which are mixtures of BODIPY ryanodine and BODIPY dehydroryanodine, most likely labeled at the 10 position of the ryanodine molecule (Figure 16.40). Structurally similar BODIPY ryanodines were shown to have a dissociation constant near that of ryanodine.^{15,17} BODIPY FL-X ryanodine has been used to visualize ryanodine receptor distribution in live porcine endothelial cells,¹⁸ in pancreatic β -cells,¹⁹ in vascular myocytes²⁰ and in the rat parotid gland.²¹

Eosin Derivatives: Inhibitors of the Calcium Pump

Eosin isothiocyanate (E-18) is a potent reversible inhibitor of the erythrocyte calcium pump, with a half-maximal inhibitory concentration of $<0.2 \mu\text{M}$.²² Eosin isothiocyanate

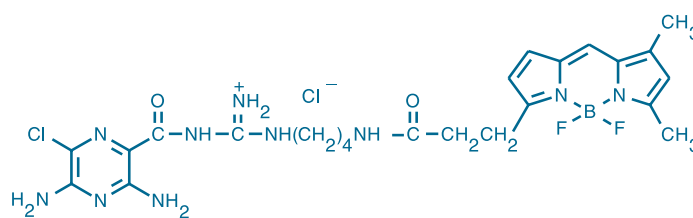


Figure 16.41 B-6905 BODIPY FL amiloride.

also reacts irreversibly at the ATP binding site in erythrocytes. Fluorescein isothiocyanate (F-143, Section 1.4) is a weaker inhibitor of this calcium pump. The succinimidyl ester of carboxyeosin diacetate (C-22803) is a cell membrane-permeant eosin derivative that inhibits the red cell plasma membrane Ca^{2+} pump.^{23,24}

Probes for Na^+ Channels and Carriers

Amiloride Analogs: Probes for the Na^+ Channel and the Na^+/H^+ Antiporter

Amiloride is a compound known to inhibit the Na^+/H^+ antiporter of vertebrate cells by acting competitively at the Na^+ -binding site.²⁵ The antiporter extrudes protons from cells using the inward Na^+ gradient as a driving force, resulting in intracellular alkalinization. In 1967, Cragoe and co-workers reported the synthesis of amiloride and several amiloride analogs, pyrazine diuretics that inhibit the Na^+ channel in urinary epithelia.²⁶ Since then, more than 1000 different amiloride analogs have been synthesized and many of these tested for their specificity and potency in inhibiting the Na^+ channel, Na^+/H^+ antiporter and $\text{Na}^+/\text{Ca}^{2+}$ exchanger.²⁷ Unmodified amiloride inhibits the Na^+ channel with an IC_{50} of less than 1 μM . We offer BODIPY FL amiloride (B-6905), a green-fluorescent probe in which the BODIPY fluorophore is attached at the R^3 position (Figure 16.41).

Additionally, amiloride is an important tool for studying the Na^+/H^+ antiporter. Structure-activity relationships have demonstrated that amiloride analogs with hydrophobic groups in the drug are the most potent and specific inhibitors for the Na^+/H^+ antiporter.²⁷⁻³² For example, our 5-(*N*-ethyl-*N*-isopropyl)-amiloride (E-3111, Figure 16.42) is 200-fold more potent than amiloride for inhibiting this antiporter.

Tetrodotoxin: Blocker of Voltage-Gated Na^+ Channels

The high-affinity neurotoxin tetrodotoxin (TTX) interferes with nerve transmission by selectively blocking the voltage-gated Na^+ channel.^{33,34} We offer a TTX preparation (T-6913, Figure



Figure 16.42 E-3111 5-(*N*-ethyl-*N*-isopropyl)amiloride, hydrochloride.

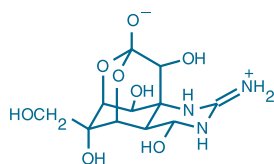


Figure 16.43 T-6913 tetrodotoxin (TTX).

16.43) that contains citrate buffer, which facilitates its dissolution in water. Our TTX is isolated in China from pufferfish and has been reported to be somewhat more active than the toxin isolated in Japan.³⁵

Cardiac Glycosides, Including Ouabain Probes for Na^+/K^+ -ATPase

Ouabain and digoxigenin are members of a class of glycosylated steroids collectively known as cardiac glycosides due to their therapeutic efficacy in the treatment of congestive heart failure. Ouabain achieves this effect by binding to the catalytic α -subunit of Na^+/K^+ -ATPase and inhibiting its transport of Na^+ across the plasma membrane. 9-Anthroyl ouabain (A-1322) — a fluorescent probe that binds to a variety of cells from different species — is useful for localizing Na^+/K^+ -ATPase and for studying its membrane orientation, mobility and dynamics.³⁶ Anthroyl ouabain has also been employed to investigate Na^+/K^+ -ATPase's active site, inhibition and conformational changes,³⁷⁻⁴² as well as to investigate the kinetics of cardiac glycoside binding.⁴³⁻⁴⁸ BODIPY FL ouabain (B-23461, Figure 16.44) and BODIPY FL digoxigenin (B-23460, Figure 16.45) offer some potentially valuable properties for these applications. Because the BODIPY FL fluorophore is nonpolar and relatively small, labeled ligands typically have superior receptor-binding properties over fluorescein- and NBD-labeled probes. BODIPY FL digoxigenin has been used to screen and sort high-affinity functional clones of single-chain Fv antibodies from a phage display library by flow cytometry.⁴⁹⁻⁵²

Probes for K^+ Channels and Carriers

Glibenclamide Probes for the ATP-Dependent K^+ Channel

Glibenclamide blocks the ATP-dependent K^+ channel, thereby eliciting insulin secretion.⁵³ In collaboration with Gabriel Haddad of Yale University, Molecular Probes has prepared the green-fluorescent BODIPY FL glibenclamide and red-fluorescent BODIPY TR glibenclamide (B-7439, B-13540; Figure 16.46) as probes for the ATP-dependent K^+ channel. Although the binding affinity of BODIPY FL glibenclamide was lower than that of the unlabeled antagonist glibenclamide, Haddad and co-workers were able to visualize ATP-dependent K^+ channels in brain sections and cultured brain cells.⁵⁴ BODIPY TR glibenclamide has been

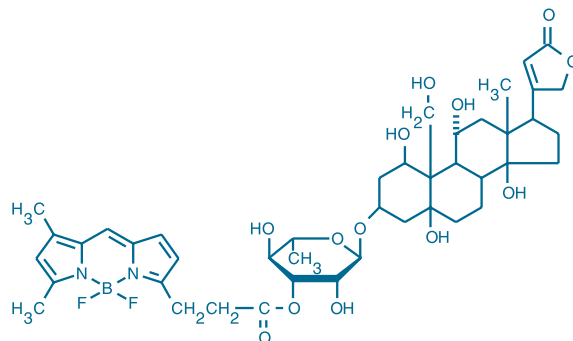


Figure 16.44 B-23461 BODIPY FL ouabain.



Our Bibliography Database Has Been Enhanced

Links to PubMed

Molecular Probes provides extensive bibliographies of published applications for most of our products. We started collecting these references in 1975 and now have more than 44,000 entries listed in our database. We have made a major enhancement to this database by linking most of the citations to the corresponding PubMed listing, including the abstract of each article (when available). This linking process has also permitted us to list all authors and full pagination for each citation. The links to PubMed are available now from any bibliography listing on our Web site.

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used to detect sulfonyleurea receptors associated with ATP-dependent K^+ channels in bovine monocytes and in β -cells^{55,56} and to label a novel mitochondrial ATP-sensitive potassium channel in brain.⁵⁷

Apamin for Small-Conductance Ca^{2+} -Activated K^+ Channels

Apamin, an 18-amino acid peptide isolated from *Apis mellifera* bee venom,^{58–61} primarily blocks small-conductance Ca^{2+} -activated K^+ channels in mammalian neurons and skeletal muscle.^{62–67} For localizing these K^+ channels, Molecular Probes has introduced Alexa Fluor 488 apamin (A-13541) and BODIPY FL apamin (A-13542). This green-fluorescent apamin derivative may be useful as an alternative to radiolabeled apamin, which has been used to study the peptide's interaction with neuroblastoma cells.⁶⁷ Apamin also possesses potent convulsant activity and is known to cross the blood–brain barrier.⁶²

Probes for Cl^- Channels and Carriers

Ivermectin Probes for Glutamate-Gated Cl^- Channels

Molecular Probes has developed a green-fluorescent derivative of the antiparasitic agent ivermectin — BODIPY FL ivermectin (B-13510). Ivermectin selectively binds to glutamate-gated Cl^- channels in invertebrate nerve and muscle cells. This binding leads to an increase in cell membrane permeability to Cl^- ions. Hyperpolarization of the cell results in paralysis and death of the parasite. BODIPY FL ivermectin has allowed researchers to investigate epithelial transport in isolated proximal kidney tubules.⁶⁸ Similar fluorescent derivatives of ivermectin have been used to measure cellular uptake of ivermectin,⁶⁹ to study ivermectin-sensitive Cl^- channels^{70,71} and to measure the concentration, mobility and distribution of ivermectin in the plasma membrane.^{72,73}

Stilbene Disulfonates: Anion-Transport Inhibitors

Molecular Probes offers five stilbene disulfonates that have been employed to inhibit (frequently irreversibly) anion transport in a large number of mammalian cell types:⁷⁴

- DBDS (D-675)
- DIDS (D-337)
- H₂DIDS (D-338)
- DNDS (D-673)
- SITS (A-339)

DNDS and SITS were among the inhibitors used to characterize three different anion exchangers in the membranes of renal brush border cells and to compare these exchangers with the band-3 anion-transport protein of erythrocyte membranes.⁷⁵ Binding of DBDS to the band-3 anion-transport protein is accompanied by a large increase in the probe's fluorescence that can be used to detect competitive binding of other drugs at this site.^{76,77}

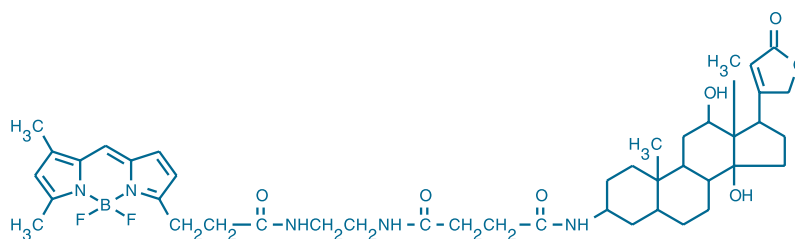


Figure 16.45 B-23460 BODIPY FL digoxigenin.

These stilbene disulfonates can, in some cases, bind specifically to proteins that are not anion transporters. For example, DIDS, H₂DIDS and SITS complex specifically with the CD4 glycoprotein on T-helper lymphocytes and macrophages, blocking HIV type-1 growth at multiple stages of the virus life cycle.⁷⁸

Our stilbene disulfonate probes, which are 95–99% pure by HPLC, have significantly higher purity and more defined composition than those available from other commercial sources.

Other Inhibitors for Anion Transporters

DiBAC₄(5)

The membrane potential-sensing dye bis-(1,3-dibutylbarbituric acid)pentamethine oxonol (DiBAC₄(5), B-436; Figure 16.47)

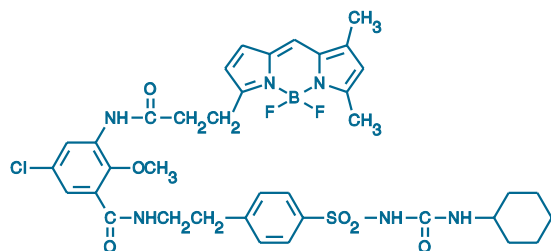


Figure 16.46 B-7439 BODIPY FL glibenclamide.

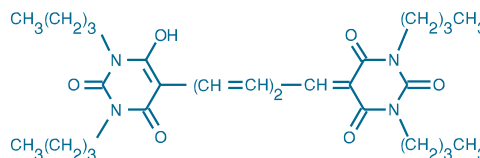


Figure 16.47 B-436 bis-(1,3-dibutylbarbituric acid)pentamethine oxonol (DiBAC₄(5)).

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The full citations and, in most cases, links to PubMed for all references in this Handbook are available at our Web site (www.probes.com/search).

Data Table — 16.3 Probes for Ion Channels and Carriers

Cat #	MW	Storage	Soluble	Abs	EC	Em	Solvent	Notes
A-339	498.45	F,DD	H ₂ O	336	47,000	436	pH 7	1
A-1322	788.89	F,D,L	DMSO	362	7,500	471	MeOH	
A-13541	~2600	F,D,L	DMSO	494	78,000	520	pH 8	2
A-13542	~2500	F,D,L	DMSO	504	83,000	513	H ₂ O	2
B-436	542.67	L	DMSO, EtOH	590	160,000	616	MeOH	3
B-6905	611.29	F,D,L	DMSO, MeOH	504	61,000	511	MeOH	
B-7431	769.18	F,D,L	DMSO, EtOH	504	74,000	511	MeOH	
B-7439	783.10	F,D,L	DMSO, EtOH	504	76,000	511	MeOH	
B-7505	880.79	FF,D,L	DMSO	504	79,000	511	MeOH	
B-13510	~1150	F,D,L	DMSO	503	71,000	511	MeOH	
B-13540	915.23	F,D,L	DMSO, EtOH	587	60,000	615	MeOH	
B-13802	1012.92	FF,D,L	DMSO	589	62,000	616	MeOH	
B-23460	805.77	F,D,L	CHCl ₃ , DMF	504	80,000	511	MeOH	
B-23461	858.74	F,D,L	DMSO	503	80,000	510	MeOH	
C-22803	873.05	F,D	DMSO	<300		none		
D-337	498.47	F,DD	H ₂ O	341	61,000	415	H ₂ O	1
D-338	500.48	F,DD	H ₂ O	286	41,000	none	MeOH	1
D-673	474.32	L	H ₂ O	352	32,000	none	H ₂ O	
D-675	622.57	L	H ₂ O	343	50,000	430	MeOH	
D-7443	686.48	F,D,L,A	DMSO, EtOH	504	83,000	511	MeOH	
E-18	704.97	F,DD,L	pH >6, DMF	521	95,000	544	pH 9	1
E-3111	336.22	D,L	H ₂ O, MeOH	378	23,000	423	MeOH	
R-7478	493.55	F,D	MeOH, DMSO	<300		none		
S-7445	760.57	F,D,L,A	DMSO, EtOH	565	143,000	570	MeOH	
T-6913	319.27	F,D	H ₂ O	<300		none		4

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

Notes

1. Isothiocyanates are unstable in water and should not be stored in aqueous solution.
2. The value of EC listed for this peptide conjugate is that of the labeling dye in free solution. Use of this value for the conjugate assumes a 1:1 dye:peptide labeling ratio and no change of EC due to dye-peptide interactions.
3. Oxonols may require addition of a base to be soluble.
4. Tetrodotoxin is unstable in alkaline solutions.

Product List — 16.3 Probes for Ion Channels and Carriers

Cat #	Product Name	Unit Size
A-339	4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid, disodium salt (SITS)	100 mg
A-1322	9-anthroyl ouabain	5 mg
A-13541	apamin, Alexa Fluor® 488 conjugate	25 µg
A-13542	apamin, BODIPY® FL conjugate	25 µg
B-436	bis-(1,3-dibutylbarbituric acid)pentamethine oxonol (DiBAC ₄ (5))	25 mg
B-6905	BODIPY® FL amiloride	25 µg
B-23460	BODIPY® FL digoxigenin	100 µg
B-7439	BODIPY® FL glibenclamide (BODIPY® FL glyburide)	100 µg
B-13510	BODIPY® FL ivermectin *mixed isomers*	100 µg
B-23461	BODIPY® FL ouabain	100 µg
B-7431	BODIPY® FL verapamil, hydrochloride	1 mg
B-7505	BODIPY® FL-X ryanodine	25 µg
B-13540	BODIPY® TR glibenclamide (BODIPY® TR glyburide)	100 µg
B-13802	BODIPY® TR-X ryanodine	25 µg
C-22803	5-(and-6)-carboxyeosin diacetate, succinimidyl ester *mixed isomers*	5 mg
D-675	4,4'-dibenzamidostilbene-2,2'-disulfonic acid, disodium salt (DBDS)	100 mg
D-338	4,4'-diisothiocyanatodihydrostilbene-2,2'-disulfonic acid, disodium salt (H ₂ DIDS)	100 mg
D-337	4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid, disodium salt (DIDS)	100 mg
D-673	4,4'-dinitrostilbene-2,2'-disulfonic acid, disodium salt (DNDS)	1 g
D-7443	DM-BODIPY® (-)-dihydropyridine *high affinity enantiomer*	25 µg
E-18	eosin-5-isothiocyanate	100 mg
E-3111	5-(<i>N</i> -ethyl- <i>N</i> -isopropyl)amiloride, hydrochloride	5 mg
R-7478	ryanodine *free of dehydroryanodine*	1 mg
S-7445	ST-BODIPY® (-)-dihydropyridine *high affinity enantiomer*	25 µg
T-6913	tetrodotoxin (TTX)	1 mg