

## Cascade Blue<sup>®</sup> and Lucifer Yellow Probes

### Fixable Polar Tracers

Molecular Probes prepares a wide variety of highly water-soluble dyes and other detectable probes that can be used as cell tracers. Polar tracers can also be incorporated into liposomes to generate polymeric fluorescent tracers or antibody-labeling reagents.<sup>1-5</sup>

In most cases, these water-soluble probes are too polar to passively diffuse through cell membranes. Consequently, special methods for loading the dyes must be employed, including microinjection, pinocytosis or techniques that temporarily permeabilize the cell's membrane. Our Influx<sup>™</sup> reagent (L-14402) efficiently loads most lucifer yellow and Cascade Blue<sup>®</sup> derivatives by a pinocytic method. Permeabilization of cells with staphylococcal  $\alpha$ -toxin or the saponin ester  $\beta$ -escin is reported to make the membrane of smooth muscle cells permeable to low molecular weight (<1000 daltons) molecules, while retaining high molecular weight compounds.<sup>6-8</sup> These reagents may facilitate the entry of many polar tracers.

### Lucifer Yellow CH

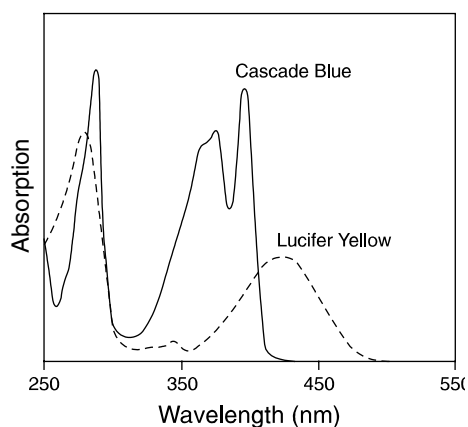
Lucifer yellow CH (LY-CH or LY) has long been a favorite tool for studying neuronal morphology because it contains a carbonylhydrazone (CH) group that allows it to be covalently linked to surrounding biomolecules during aldehyde fixation.<sup>9,10</sup> Loading of this polar tracer and other similar impermeant dyes is usually accomplished by microinjection,<sup>11</sup> pinocytosis,<sup>12</sup> scrape loading,<sup>13</sup> ATP-induced permeabilization<sup>14</sup> or osmotic shock.<sup>15</sup> Lucifer yellow CH localizes in the plant vacuole when taken up either through what is thought to be anion transport channels<sup>16</sup> or by fluid-phase endocytosis.<sup>17</sup> The lithium salt of lucifer yellow CH<sup>18</sup> (L-453) is widely used for microinjection because of its high water solubility (~8%). We also supply a sterile-filtered 100 mM (~4.6%) solution of this salt in water (L-12926). The potassium

salt (L-1177, solubility ~1%) or the ammonium salt (L-682, solubility ~6%) may be preferred in applications where lithium ions interfere with biological function.

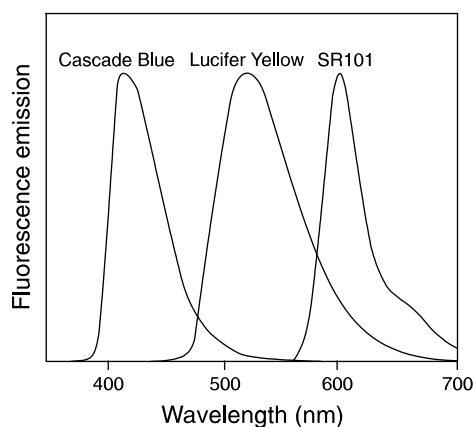
Although its low absorbance at 488 nm ( $\epsilon \sim 700 \text{ cm}^2 \text{ M}^{-1}$ ) makes it inefficiently excited with the argon-ion laser, lucifer yellow CH has been used as a neuronal tracer in confocal laser scanning microscopy studies.<sup>19,21</sup> For electron microscopy studies, lucifer yellow can be used to photoconvert diaminobenzidine (DAB) into an insoluble, electron-dense reaction product.<sup>22-24</sup> Alternatively, anti-lucifer yellow dye antibodies may be used with enzyme-mediated immunohistochemical methods to develop a more permanent, fade-free colorimetric or electron-dense signal from dye-filled neurons that is suitable for light or electron microscopy.<sup>25-29</sup>

### Cascade Blue Hydrazide

Molecular Probes' Cascade Blue hydrazide is a fixable analog of the blue fluorescent tracer methoxyphenylsulfonic acid (MPTS, M-395).<sup>30</sup> All of the Cascade Blue hydrazide derivatives have good water solubility, ~1% for the sodium and potassium salts (C-687, C-3221) and ~8% for the lithium salt (C-3239). They also exhibit a higher absorbance ( $\epsilon_{400 \text{ nm}} > 28,000 \text{ cm}^2 \text{ M}^{-1}$ ) and quantum yield (~0.54 in water) than lucifer yellow CH. In addition, Cascade Blue derivatives have good photostability and emissions that are well resolved from those of fluorescein and lucifer yellow CH.<sup>31</sup> Cascade Blue hydrazide and lucifer yellow CH can be simultaneously excited at <410 nm (Figure 1) for two-color detection at about 430 and 530 nm. Although a standard DAPI/Hoechst optical filter set can be used with Cascade Blue conjugates, the fluorescence will be brighter when viewed through optical filters optimized for the spectral properties of the Cascade Blue dye (ex/em = 400/420 nm). Cascade Blue hydrazide, lucifer yellow CH and sulforhodamine 101 can be used in combination for three-color mapping of neuronal processes (Figure 2).



**Figure 1.** Absorption spectra for equal concentrations of Cascade Blue hydrazide (C-687) and lucifer yellow CH (L-453) in water.



**Figure 2.** Normalized fluorescence emission spectra for Cascade Blue hydrazide (C-687), lucifer yellow CH (L-453) and sulforhodamine 101 (S-359) in water.

Molecular Probes also offers anti-Cascade Blue dye antibodies for localizing Cascade Blue dye-filled cells following fixation. Like lucifer yellow CH, Cascade Blue hydrazide and some other polar tracers are taken up by plants and sequestered into their central vacuoles. In onion epidermal cells, this uptake is blocked by probenecid, indicating that transfer may be through anion-transport channels.<sup>16</sup>

In addition to its use for preparing protein conjugates, the membrane-impermeant Cascade Blue acetyl azide may be useful for identifying proteins located on extracellular cell surfaces. Molecular Probes offers fixable Cascade Blue derivatives for use as polar tracers and Cascade Blue conjugates of a variety of proteins, nucleotides and dextrans, as well as antibodies directed against the Cascade Blue fluorophore.

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## Other Lucifer Yellow and Cascade Blue Tracers

### Lucifer Yellow Derivatives

Like lucifer yellow CH, our lucifer yellow ethylenediamine (A-1339) and lucifer yellow cadaverine are fixable with standard aldehyde fixatives and can be used as building blocks for new lucifer yellow derivatives.<sup>32</sup> The photoreactive lucifer yellow azide (lucifer yellow AB, A-629) has been used to photolabel cytoplasmic and luminal domains of Ca<sup>2+</sup>-ATPase in sarcoplasmic reticulum vesicles.<sup>33</sup> The thiol-reactive lucifer yellow iodoacetamide (L-1338) can also be used as a microinjectable polar tracer, as well as for preparing fluorescent liposomes<sup>1</sup> and for detecting the accessibility of thiols in membrane-bound proteins.<sup>34,35</sup> In addition to these lucifer yellow derivatives, we offer lucifer yellow-conjugated dextrans (D-1825).

### Cascade Blue Derivatives

Cascade Blue acetyl azide (C-2284) is a water-soluble, amine-reactive tracer that can be introduced either by microinjection or by fusion of dye-filled liposomes with cells. It is also the reactive form of the Cascade Blue dye that Molecular Probes uses to prepare the biomolecule conjugates. Once inside the cell, this Cascade Blue derivative will react with the amine groups of intracellular proteins. Cascade Blue ethylenediamine and cadaverine (C-621, C-622) are aldehyde-fixable fluorophores similar to the amine derivatives of lucifer yellow (A-1339). The photoreactive Cascade Blue azide derivative is a photoaffinity label analogous to lucifer yellow azide (A-629) that can be microinjected into cells and subsequently fixed in place by UV illumination. Cascade Blue dye-labeled dextrans are also available (D-1976, D-7132).

### Fluorescent Biotin Derivatives

Fluorescence of the finer processes of dye-filled neurons may fade rapidly or be obscured by the more intensely stained portions of the neuron, necessitating further amplification of the signal or ultrastructural detection methods. Cascade Blue biocytin and lucifer yellow biocytin (L-6950) incorporate a fluorophore, biotin and a fixable primary amine into a single molecule, thus enabling researchers to amplify the signals of these tracers with fluorescent or enzyme-labeled avidin or streptavidin conjugates. Although our lucifer yellow cadaverine biotin-X (L-2601) lacks a primary amine, it was reported that this tracer was well retained in aldehyde-fixed tissues, even after sectioning, extraction with detergents and several washes.<sup>36,37</sup> Because lucifer yellow biocytin and Cascade Blue biocytin contain free primary amines, they should be even more efficiently fixed by formaldehyde or glutaraldehyde.

Fluorescent derivatives of biotin should also be useful for directly localizing biotin-binding proteins.<sup>38</sup> Fluorescein biotin (B-1370) is a nonfixable fluorescent biotin derivative developed by Molecular Probes as an alternative to radioactive biotin for detecting and quantitating biotin binding sites by either fluorescence or absorbance. A fluorescence polarization assay that employs competitive binding of fluorescein biotin to assess the degree of biotinylation of proteins has recently been reported.<sup>39</sup>

### Anti-Lucifer Yellow Dye and Anti-Cascade Blue Dye Antibodies

Molecular Probes' anti-lucifer yellow dye antibodies were specifically developed to overcome the limitations of lucifer yellow CH, an aldehyde-fixable fluorescent cell tracer that has long been used by neuroscientists to identify patterns of gap junctional communication,<sup>9</sup> to assay the outgrowth of developing neurons<sup>40</sup> and to characterize the morphology of neurons from which electrical recordings have been made.<sup>11</sup> Even though the cell soma of a lucifer yellow dye-filled neuron may be brightly stained, its finer processes can sometimes be faint and may fade rapidly or be obscured by the more intensely stained portions of the neuron. Investigators have been able to overcome these limitations by using anti-lucifer yellow dye antibodies in conjunction with standard enzyme-mediated immunohistochemical methods to develop a more permanent, fade-free colorimetric signal for light microscopy.<sup>26,27,29,41</sup> Anti-lucifer yellow dye antibodies have also been used to develop tissue for electron microscopy<sup>42</sup> and to distinguish neurons filled with lucifer yellow CH from those injected with the lectin *Phaseolus vulgaris* leucoagglutinin<sup>28</sup> (PHA-L). For these applications, Molecular Probes offers unconjugated and biotinylated rabbit polyclonal anti-lucifer yellow dye (A-5750, A-5751) and anti-Cascade Blue dye (A-5760) antibodies.

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

Cat #	Product Name	Unit Size
A-1339	N-(2-aminoethyl)-4-amino-3,6-disulfo-1,8-naphthalimide, dipotassium salt (lucifer yellow ethylenediamine).....	25 mg
A-5760	anti-Cascade Blue <sup>®</sup> , rabbit IgG fraction *3 mg/mL* .....	0.5 mL
A-5750	anti-lucifer yellow, rabbit IgG fraction *3 mg/mL* .....	0.5 mL
A-5751	anti-lucifer yellow, rabbit IgG fraction, biotin-XX conjugate *3 mg/mL* .....	0.5 mL
A-2663	avidin, NeutrAvidin <sup>™</sup> , Cascade Blue <sup>®</sup> conjugate.....	1 mg
C-2284	Cascade Blue <sup>®</sup> acetyl azide, trisodium salt .....	5 mg
C-621	Cascade Blue <sup>®</sup> ethylenediamine, trisodium salt .....	10 mg
C-3239	Cascade Blue <sup>®</sup> hydrazide, trilithium salt.....	10 mg
C-3221	Cascade Blue <sup>®</sup> hydrazide, tripotassium salt .....	10 mg
C-687	Cascade Blue <sup>®</sup> hydrazide, trisodium salt .....	10 mg
C-7612	ChromaTide <sup>™</sup> Cascade Blue <sup>®</sup> -7-dUTP (Cascade Blue <sup>®</sup> -7-dUTP) *1 mM in buffer* .....	25 µL
D-7132	dextran, Cascade Blue <sup>®</sup> , 3000 MW, anionic, lysine fixable .....	10 mg
D-1976	dextran, Cascade Blue <sup>®</sup> , 10,000 MW, anionic, lysine fixable .....	25 mg
D-1825	dextran, lucifer yellow, 10,000 MW, anionic, lysine fixable .....	25 mg
I-14402	Influx <sup>™</sup> pinocytic cell-loading reagent *makes 10 x 5 mL* .....	1 set
L-6950	lucifer yellow biocytin, potassium salt (biocytin lucifer yellow) .....	5 mg
L-2601	lucifer yellow cadaverine biotin-X, dipotassium salt.....	10 mg
L-682	lucifer yellow CH, ammonium salt.....	25 mg
L-453	lucifer yellow CH, lithium salt.....	25 mg
L-12926	lucifer yellow CH, lithium salt *for microinjection* *100 mM in water* .....	100 µL
L-1177	lucifer yellow CH, potassium salt .....	25 mg
L-1338	lucifer yellow iodoacetamide, dipotassium salt .....	25 mg
M-395	8-methoxyppyrene-1,3,6-trisulfonic acid, trisodium salt (MPTS) .....	100 mg

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