Dextran Conjugates

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light

Ex/Em: See Tables 1 and 2

Introduction

Dextrans—hydrophilic polysaccharides synthesized by *Leuconostoc* bacteria—are characterized by their high molecular weight, good water solubility, low toxicity, and relative inertness. These properties make dextrans effective water-soluble carriers for dyes, indicators, and reactive groups in a wide variety of applications. Moreover, their biologically uncommon α-1,6-polyglucose linkages are resistant to cleavage by most endogenous cellular glycosidases; therefore dextran conjugates make ideal long-term tracers for live cells. Fluorescent dextrans also serve as valuable markers for cell loading of macromolecules by microinjection,[1-3] vesicular fusion,[4] and electroporation,[5] as well as for the uptake and internal processing of exogenous materials by phagocytotic and endocytic pathways.[6,7]

Molecular Probes offers numerous dextran conjugates in a variety of molecular weights and labeled with a wide array of substituents, including our superior Alexa Fluor® dyes (see Tables 1 and 2). In addition, we prepare a variety of "lysine-fixable" probes which have lysine residues incorporated into the dextran conjugate. These lysinated dextrans are useful for applications that require the dextran tracer subsequently be treated with aldehyde fixatives for analysis.[8]

Storage and Handling

Materials Provided

Molecular Probes' dextran conjugates are provided as pure lyophilized powder containing only trace amounts of salts. Purification of the conjugates is performed using a combination of precipitation, dialysis, gel filtration and other techniques. The conjugates are then assayed by thin-layer chromatography (TLC) to ensure absence of free dye. Although these conjugates are quite stable, we recommend that the powder be stored at ≤–20°C and protected from light.

Table 1. Spectral characteristics of Molecular Probes' dye-labeled dextran conjugates.

Conjugate	Ex (nm) *	Em (nm) *
Cascade Blue®	400	420
Lucifer Yellow	425	528
HPTS	469	530
Fluorescein	494	521
Alexa Fluor® 488	495	519
Oregon Green® 488	496	524
Rhodamine Green™	502	527
BODIPY® FL	505	513
Oregon Green® 514	511	530
JOE †	525	555
Carboxy-Q-Rhodamine	545	575
TMR ‡	555	580
Alexa Fluor® 546	556	573
Alexa Fluor® 555	555	565
Rhodamine B	570	590
Alexa Fluor® 568	578	603
Alexa Fluor® 594	590	617
Texas Red®	595	615
Alexa Fluor® 647	650	668
Alexa Fluor® 680	679	702

***** Approximate fluorescence excitation and emission maxima, in nm.
† JOE = 4′,5′-dichloro-2′,7′-dimethoxyfluorescein. **‡** TMR = tetramethylrhodamine.

Making and Storing Solutions

Dextran conjugates are generally soluble in aqueous buffers to at least 10 mg/mL. Their solubilities decrease as the molecular weight increases; thus the maximum solubility in aqueous buffer is about 100 mg/mL for 3,000 MW dextrans, 50 mg/mL for 10,000 MW dextrans, 25 mg/mL for 70,000 MW dextrans and 5–10 mg/mL for the 500,000 and 2,000,000 MW dextrans. Dextrans with hydrophobic functional groups (e.g., rhodamine or biotin) may be more readily soluble in slightly alkaline (pH 8 or above) buffers. Vortexing, sonicating, or briefly heating (40–50°C) may increase the solubility. Any insoluble particles formed during dissolution should be removed by centrifuging the mixture in a microfuge at 12,000 × g for 5 minutes. Alternatively, the particles may be removed by filtrating a dilute solution of the dextran through Whatman #2 filter paper. Aqueous solutions of dextrans may be stored at 2–6°C for several weeks, with the addition of

Table 2. Spectral characteristics of Molecular Probes' pH and Ca^{2+} indicator dextrans.

Conjugate	Ex (nm) *	Em (nm) *
LysoSensor™ Yellow/Blue †	335, 381	452, 521
Indo ‡	356, 343	466, 408
Fura ‡	364, 340	503, 495
Fluo-4	494	516
Oregon Green® 488 BAPTA-1	496	524
Calcium Green™-1	505	532
BCECF	508	532
DM-NERF	517	540
Cl-NERF	519	544
Rhod	530	576
X-rhod	580	602
SNARF®-1	563	639

* Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. † Dual-excitation and dual-emission maxima, sensitive to pH. ‡ Ratioable Ca^{2+} indicators, Ex/Em values are given for the zero and saturating levels of the ion.

sodium azide to a final concentration of 2 mM to inhibit bacterial growth. Sterilization of fairly dilute dextran solutions (1–10 mg/mL) may be performed by filtration using 0.2 µm pore-diameter sterile filters. For long-term storage, divide the aqueous solution into aliquots and freeze at ≤–20°C. AVOID REPEATED FREEZING AND THAWING.

Characteristics

Molecular Weight

We offer dextrans with nominal molecular weights (MW) of 3,000; 10,000; 40,000; 70,000; 500,000; and 2,000,000 daltons. Because unlabeled dextrans are polydisperse—and may become more so during the chemical processes required for their modification and purification—the actual molecular weights present in a particular sample may have a broad distribution. For example, our 3,000 MW dextran preparations contain polymers with molecular weights predominantly in the range of ~1,500–3,000 daltons, including the dye or other label, while our 70,000 MW dextran preparations contain polymers with molecular weights ranging from 60,000 to 90,000 daltons.

Degree of Labeling

The degree of labeling of the dextran conjugate is indicated on the container's label. Typically this value is one-half to one dye per dextran in the 3,000 MW range; one to two dyes per dextran in the 10,000 MW range; two to six dyes per dextran in the 40,000 MW range; three to eight dyes per dextran in the 70,000 MW range; and 13–130 dyes per dextran in the 500,000 and 2,000,000 MW ranges. The degree of labeling is optimized to provide the brightest conjugate, without producing quenching effects or undesired interactions with cellular components that can occur with excessive labeling. Molecular Probes' fluorescent dextran conjugates are consistently more fluorescent than those available from other sources.

Net Charge and Method of Labeling

The net charge on a dextran depends on the fluorophore and the method of preparation. Molecular Probes prepares most of its dextrans by reacting a water-soluble amino dextran with the succinimidyl ester (rather than the isothiocyanate) derivative of the appropriate dye. This method provides selectivity, stability, and low toxicity. Succinimidyl esters are quite selective toward aliphatic amines, whereas isothiocyanates will also react with alcohols and other functional groups. Moreover, the activated esters yield an amide linkage, which is somewhat more stable than the corresponding thiourea bond formed with isothiocyanates.

Once the dye has been added, the unreacted amines on the dextran are usually capped to yield a neutral or anionic dextran. Thus the net charge on the dextran depends both on the attached fluorophore and on the capping reagent used. In most cases the resulting conjugate is anionic; exceptions are the zwitterionic rhodamine and Texas Red® fluorophores, which result in essentially neutral conjugates. For Rhodamine Green™ dextrans, the unreacted amines are not capped after dye conjugation. In this case the dextran conjugates are either neutral or cationic.

Range of Substituents

Molecular Probes' selection of fluorophores used in our labeled dextrans is summarized in Table 1, while our pH and Ca^{2+} indicator conjugates are listed in Table 2. In addition we offer amino-modified and biotinylated dextrans, the latter of which can be detected with our wide array of avidin and streptavidin probes (see Molecular Probes' *The Handbook: A Guide to Fluorescent Probes and Labeling Technologies,* at our website, probes.invitrogen.com. .

Applications of Labeled Dextrans

Size Exclusion

The availability of various sizes of conjugated dextrans makes them well suited for evaluating exclusion properties of artificial polymer matrices,[9] vascular networks, [10-13] and cell membranes.[14-16] The size-exclusion properties of dextrans have also been exploited to investigate intracellular communication through gap junctions.[17-19]

Cell Lineage

Dextran conjugates have been used extensively by developmental biologists for tracing cell lineage because of their excellent retention properties and low toxicity.[20-25] Of particular interest to the developmental biologist may be dextran conjugates with a caged fluorophore.[26] Such dextrans can be injected early in development when the cells are large and then later activated in the growing embryo when the cells of interest may be small and buried deep within the tissue.

Neuronal Tracing

Dextran conjugates with molecular weights up to 70,000 daltons are routinely employed to trace neuronal projections and can function efficiently as anterograde or retrograde tracers, depending on the study method and tissue type used.[27,28] Our 3,000 MW dextran conjugates may be preferred in some applications because they penetrate peripheral neuronal processes better and diffuse faster than higher-MW dextrans.[29,30]

Endocytosis

Fluorescent dextran conjugates have been used to monitor the uptake and internal processing of exogenous materials by endocytosis.[31-35] Our dextran conjugates may also be useful for studies of endosome fusion,[36] cell membrane changes, [37] and vesicular morphology.[38]

Fluid Dynamics

Fluorescent dextrans have been used in studies of macromolecular diffusion through cytoplasm,[39] fluid flow velocity,[40] liposome encapsulation, [41] and vascular flow in whole animals.[42-44] Monitoring the diffusion of the dextran tracer is typically accomplished using fluorescence recovery after photobleaching (FRAP) techniques,[45] or by photoactivation of caged fluorophore–dextran conjugates.[46-48]

Intracellular Ion Measurement

Compared to the free ion indicator, dextran conjugates of these same indicators exhibit both reduced compartmentalization and much lower rates of dye leakage. Such conjugates have been utilized in measurements of intracellular pH,[49-51] as well as Ca^{2+} and/or Mg^{2+} concentrations in a wide variety of cell types, including plant and fungal cells.[52-54]

Experimental Protocols

Since the molecular weight of the dextran, net charge, degree of labeling, and nature of the dye may affect the experiment, researchers are advised to consult the primary literature for information specific to the application of interest. As an aid, Molecular Probes has compiled a dextran bibliography containing over 950 references that cover a wide variety of applications. This bibliography can be accessed through our website (probes.invitrogen.com); it can be found in our product list under the number D8998.

References

1. J Biochem Biophys Methods 16, 283 (1988); **2.** Proc Natl Acad Sci USA 85, 6748 (1988); **3.** J Cell Biol 114, 967 (1991); **4.** J Biol Chem 268, 1430 (1993); **5.** Anal Biochem 194, 198 (1991); **6.** Proc Natl Acad Sci USA 75, 3327 (1978); **7.** J Cell Biol 85, 660 (1980); **8.** Science 252, 1173 (1991); **9.** J Pharm Pharm-acol 43, 275 (1991); **10.** Am J Physiol 245, H495 (1983); **11.** Arch Ophthalmol 105, 844 (1987); **12.** Exp Eye Res 24, 607 (1977); **13.** Histochemistry 71, 209 (1981); **14.** J Biol Chem 267, 18424 (1992); **15.** Anal Biochem 194, 418 (1991); **16.** Biochim Biophys Acta 1022, 381 (1990); **17.** J Cell Biol 113, 371 (1991); **18.** Development 112, 431 (1991); **19.** Devel Biol 144, 47 (1991); **20.** Science 252, 569 (1991); **21.** Development 108, 581 (1990); **22.** Nature 344, 431 (1990); **23.** Dev Biol 124, 269 (1987); **24.** Dev Biol 108, 78 (1985); **25.** Dev Biol 108, 86 (1985); **26.** Bioorg Med Chem Lett 11, 2181 (2001); **27.** Brain Res 526, 127 (1990); **28.** Brain Res Bull 51, 11 (2000); **29.** J Cell Biol 102, 2015 (1986); **30.** J Cell Biol 102, 1183 (1986); **31.** J Biol Chem 269, 12918 (1994); **32.** Am J Physiol 258, C309 (1990); **33.** J Cell Physiol 131, 200 (1987); **34.** Proc Natl Acad Sci USA 82, 8523 (1985); **35.** Exp Cell Res 150, 36 (1984); **36.** Biophys J 69, 716 (1995); **37.** Cytometry 14, 826 (1993); **38.** J Biol Chem 269, 19005 (1994); **39.** J Neurosci 12, 77 (1992); **40.** Microvasc Res 43, 235 (1992); **41.** Cell 64, 393 (1991); **42.** Life Sci 49, 1699 (1991); **43.** J Dev Physiol 10, 227 (1988); **44.** Proc Soc Exp Biol Med 161, 370 (1979); **45.** Biophys J 35, 351 (1981); **46.** Cell 68, 923 (1992); **47.** Nature 352, 126 (1992); **48.** J Cell Biol 109, 637 (1989); **49.** J Cell Biol 101, 2302 (1985); **50.** J Biol Chem 265, 7645 (1990); **51.** J Histochem Cytochem 38, 685 (1990); **52.** Science 258, 292 (1992); **53.** Proc Natl Acad Sci USA 89, 3591 (1992); **54.** J Microsc 166, 57 (1992).

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

Cat #	Product Name	Unit Size
C6765	Calcium Green™-1 dextran, potassium salt, 3000 MW, anionic	5 mg
C3713	Calcium Green™-1 dextran, potassium salt, 10,000 MW, anionic	5 mg
C3714	Calcium Green™-1 dextran, potassium salt, 70,000 MW, anionic	5 mg
C6766	Calcium Green™-1 dextran, potassium salt, 500,000 MW, anionic	5 mg
D34682	dextran, Alexa Fluor® 488; 3000 MW, anionic	2 mg
D22910	dextran, Alexa Fluor® 488; 10,000 MW, anionic, fixable	5 mg
D22911	dextran, Alexa Fluor® 546; 10,000 MW, anionic, fixable	5 mg
D34679	dextran, Alexa Fluor® 555; 10,000 MW, anionic, fixable	5 mg
D22912	dextran, Alexa Fluor® 568; 10,000 MW, anionic, fixable	5 mg
D22913	dextran, Alexa Fluor® 594; 10,000 MW, anionic, fixable	5 mg
D22914	dextran, Alexa Fluor® 647; 10,000 MW, anionic, fixable	2 mg
D34681	dextran, Alexa Fluor® 680; 3000 MW, anionic	2 mg
D34680	dextran, Alexa Fluor® 680; 10,000 MW, anionic, fixable	5 mg
D3330	dextran, amino, 3000 MW	100 mg
D1860	dextran, amino, 10,000 MW	1 g
D1861	dextran, amino, 40,000 MW	1 g
D1862	dextran, amino, 70,000 MW	1 g
D7144	dextran, amino, 500,000 MW	100 mg
D1878	dextran, BCECF, 10,000 MW, anionic	10 mg
D1880	dextran, BCECF, 70,000 MW, anionic	10 mg
D7134	dextran, biotin, 3000 MW, neutral	10 mg
D7135	dextran, biotin, 3000 MW, lysine fixable (BDA-3000)	10 mg
D1956	dextran, biotin, 10,000 MW, lysine fixable (BDA-10,000)	25 mg
D1957	dextran, biotin, 70,000 MW, lysine fixable (BDA-70,000)	25 mg
D7142	dextran, biotin, 500,000 MW, lysine fixable (BDA-500,000)	10 mg

D7168	dextran, BODIPY® FL, 10,000 MW, fixable	5 mg
D34678	dextran, 5-(and-6)-carboxy-Q-rhodamine, CMNCBZ-caged, 10,000 MW, anionic	5 mg
D7132	dextran, Cascade Blue®, 3000 MW, anionic, lysine fixable	10 mg
D1976	dextran, Cascade Blue®, 10,000 MW, anionic, lysine fixable	25 mg
D12980	dextran, 4′,5′-dichloro-2′,7′-dimethoxyfluorescein, 10,000 MW, anionic	5 mg
D3305	dextran, fluorescein, 3000 MW, anionic	10 mg
D3306	dextran, fluorescein, 3000 MW, anionic, lysine fixable	10 mg
D1820	dextran, fluorescein, 10,000 MW, anionic, lysine fixable (fluoro-emerald)	25 mg
D1821	dextran, fluorescein, 10,000 MW, anionic	25 mg
D1844	dextran, fluorescein, 40,000 MW, anionic	25 mg
D1845	dextran, fluorescein, 40,000 MW, anionic, lysine fixable	25 mg
D1822	dextran, fluorescein, 70,000 MW, anionic, lysine fixable	25 mg
D1823	dextran, fluorescein, 70,000 MW, anionic	25 mg
D7136	dextran, fluorescein, 500,000 MW, anionic, lysine fixable	10 mg
D7137	dextran, fluorescein, 2,000,000 MW, anionic, lysine fixable	10 mg
D7156	dextran, fluorescein and biotin, 3000 MW, anionic, lysine fixable (micro-emerald)	5 mg
D7178	dextran, fluorescein and biotin, 10,000 MW, anionic, lysine fixable (mini-emerald)	10 mg
D1950	dextran, fluorescein and tetramethylrhodamine, 10,000 MW, anionic	10 mg
D1951	dextran, fluorescein and tetramethylrhodamine, 70,000 MW, anionic	10 mg
D7179	dextran, 8-hydroxypyrene-1,3,6-trisulfonic acid, 10,000 MW, anionic (HPTS dextran)	5 mg
D1825	dextran, lucifer yellow, 10,000 MW, anionic, lysine fixable	25 mg
D7170	dextran, Oregon Green® 488; 10,000 MW, anionic	5 mg
D7171	dextran, Oregon Green® 488; 10,000 MW, anionic, lysine fixable	5 mg
D7172	dextran, Oregon Green® 488; 70,000 MW, anionic	5 mg
D7173	dextran, Oregon Green® 488; 70,000 MW, anionic, lysine fixable	5 mg
D7174	dextran, Oregon Green® 514; 10,000 MW, anionic	5 mg
D7176	dextran, Oregon Green® 514; 70,000 MW, anionic	5 mg
D1824	dextran, rhodamine B, 10,000 MW, neutral	25 mg
D1841	dextran, rhodamine B, 70,000 MW, neutral	25 mg
D7163	dextran, Rhodamine Green™, 3000 MW	5 mg
D7153	dextran, Rhodamine Green™, 10,000 MW, lysine fixable	10 mg
D3303	dextran, SNARF®-1, 10,000 MW, anionic	5 mg
D3304	dextran, SNARF®-1, 70,000 MW, anionic	5 mg
D3307	dextran, tetramethylrhodamine, 3000 MW, anionic	10 mg
D3308	dextran, tetramethylrhodamine, 3000 MW, anionic, lysine fixable	10 mg
D1868	dextran, tetramethylrhodamine, 10,000 MW, anionic, fixable	25 mg
D1817	dextran, tetramethylrhodamine, 10,000 MW, lysine fixable (fluoro-ruby)	25 mg
D1816	dextran, tetramethylrhodamine, 10,000 MW, neutral	25 mg
D1842	dextran, tetramethylrhodamine, 40,000 MW, neutral	25 mg
D1818	dextran, tetramethylrhodamine, 70,000 MW, lysine fixable	25 mg
D1819	dextran, tetramethylrhodamine, 70,000 MW, neutral	25 mg
D7139	dextran, tetramethylrhodamine, 2,000,000 MW, lysine fixable	10 mg
D3312	dextran, tetramethylrhodamine and biotin, 10,000 MW, lysine fixable (mini-ruby)	10 mg
D7162	dextran, tetramethylrhodamine and biotin, 3000 MW, lysine fixable (micro-ruby)	5 mg
D3328	dextran, Texas Red®, 3000 MW, lysine fixable	10 mg
D3329	dextran, Texas Red®, 3000 MW, neutral	10 mg
D1863	dextran, Texas Red®, 10,000 MW, lysine fixable	25 mg
D1828	dextran, Texas Red®, 10,000 MW, neutral	25 mg
D1829	dextran, Texas Red®, 40,000 MW, neutral	25 mg
D1864	dextran, Texas Red®, 70,000 MW, lysine fixable	25 mg
D1830	dextran, Texas Red®, 70,000 MW, neutral	25 mg
F36250	fluo-4 dextran, potassium salt, 10,000 MW, anionic (high-affinity version)	5 mg
F14240	fluo-4 dextran, potassium salt, 10,000 MW, anionic (low-affinity version)	5 mg
F3029	fura dextran, potassium salt, 10,000 MW, anionic	5 mg
I3032	indo dextran, potassium salt, 10,000 MW, anionic	5 mg
I3033	indo dextran, potassium salt, 70,000 MW, anionic	5 mg
L22460	LysoSensor™ Yellow/Blue dextran, 10,000 MW, anionic, fixable	5 mg
O6798	Oregon Green® 488 BAPTA-1 dextran, potassium salt, 10,000 MW, anionic	5 mg
R34676	rhod dextran, potassium salt, 10,000 MW, anionic (high-affinity version)	5 mg
R34677	rhod dextran, potassium salt, 10,000 MW, anionic (low-affinity version)	5 mg
X34675	X-rhod dextran, potassium salt, 10,000 MW, anionic	5 mg

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