Labeled Dextran Conjugates

Labeled dextran are used typically as long-term tracers for live cells studies. The lysinated dextrans are useful for applications that require the dextran tracer subsequently be treated with aldehyde fixatives for analysis.

Product Information

Product name cat. number Anionic form	MW g.mol-1	$\lambda_{exc./em.}$ nm	Product name cat. number Anionic form
Dextran–biotin			**Dextran-biotin, Lysine Fixable**
	3 000		FP-66276B, 5 mg
	10 000		FP-18938B, 10 mg
	40 000		FP-B4CPX0, 10 mg
FP-BN454B, 10 mg	70 000		
	500 000		
Dextran-amine			can be used to react with other chemical groups via standard chemistry (e.g. EDC-mediated reductive amidation with carboxyls)
FP-57615A, 100mg	3 000		
FP-AQ9XI1, 100mg	5 000		
FP-AQ9XJ1, 100mg	10 000		
FP-11739A, 100mg FP-11739B, 1 g	40 000		
FP-11740A, 100mg FP-11740B, 1g	70 000		
FP-0B3261, 100 mg	500 000		
FP-AQ9XL1, 100mg	2 000 000		
Dextran-Blue (Cibacron blue F3GA)		621.5 / -	
FP-1J1090, 1 g	5 000		
FP-1J1100, 1 g	10 000		
FP-BSEMM0, 1 g	40 000		
FP-1J1120, 10 g	70 000		
FP-1J1140, 10 g	110 000		
FP-1J1151, 1 g FP-1J1150, 10 g	500 000		
FP-1J1161, 1 g FP-1J1160, 10 g	2 000 000		
Dextran–FITC		494/518	**Dextran-FITC, Lysine Fixable**
FP-67369A, 10 mg	3 000		FP-66264A, 10 mg
FP-CL3200, 100 mg	4 000		
FP-CL3210, 100 mg FP-87954A, 25 mg	10 000		FP-86710A, 10 mg
FP-CL3220, 100 mg	20 000		
FP-36223C, 100 mg FP-90231A, 30 mg	40 000		
FP-90231B, 100 mg FP-90231A, 30 mg	70 000		
FP-CL3230, 100 mg FP-WT8720, 10mg	500 000		FP-56557A, 10 mg
Dextran–Rhodamine B (TRITC)		555/580	**Dextran- Rhodamine B, Lysine Fixable**
FP-BV1390, 10 mg	3 000		FP-14440A, 10 mg
FP-CL3150, 100 mg	4 000		FP-BSEPC0, 10 mg
FP-319441, 25 mg	10 000		FP-91830B, 30 mg (Fluoro-Ruby)
FP-04788A, 30 mg	40 000		
FP-49674A, 30 mg	70 000		FP-69803A, 10 mg
FP-BV2850, 10 mg	500 000		
Dextran-CY$_{anine}$5		580/675	
FP-WT8780, 2 mg	3 000		
FP-WT8790, 5 mg	10 000		
FP-WT8800, 5 mg	40 000		
FP-WT8810, 5 mg	70 000		
FP-WT8820, 5 mg	500 000		

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Product name cat. number Anionic form	MW g.mol-1	$\lambda_{exc./em.}$ nm	Product name cat. number Anionic form
Dextran-CY$_{anine}$5.5		663/690	
FP-DZ0571, 2 mg	3 000		
FP-DZ0581, 5 mg	10 000		
FP-DZ0591, 5 mg	40 000		
FP-DZ0601, 5 mg	70 000		
FP-DZ0611, 5 mg	500 000		
Dextran-CY$_{anine}$7		743/767	
FP-DZ0641, 5 mg	8 000		

Storage: +4°C (K)

Introduction

Dextrans are naturally synthesized polysaccharides with good water solubility, low toxicity and varied molecular weights. They have been successfully used as long-term tracers for live cells.
Our dextrans were purified by size chromatography to remove non-conjugated molecules and ensure adequate cell biology assays applications. Dextrans were functionalized with a variety of groups, dyes and other biomolecules.

Technical and scientific information

Dextrans features

Dextrans, are hydrophilic polysaccharides synthesized by *Leuconostoc* bacteria. They have high molecular weight, and after fractionation with in a variety of sizes. Dextrans have good water solubility, low toxicity and relative inertness. Their α-1,6-poly-glucose linkages are resistant to cleavage by most endogenous cellular glycosidases. These properties make Dextrans serve as effective water-soluble carriers for dyes, indicators and reactive groups in a wide variety of applications, especially as long-term tracers for live cells.

Numerous dextran conjugates in a variety of molecular weights (3 000, 10 000, 40 000, 70 000, 500 000) and labeled with a wide array of substituents are available.

Unlabeled dextrans are polydisperse, and may become more upon chemical processes required for their modification and purification. The actual molecular weights present in a particular sample may have a broad distribution : for example, the 3 000 MW dextran preparations contain polymers with molecular weights predominantly in the range of ~1500-3 000 daltons, including the dye or other label, while the 70 000 MW dextran preparations contain polymers with molecular weights ranging from 60 000 to 90 000 daltons.

The **degree of labeling** of the dextran conjugate 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.
Our labeling and purification process provides improved selectivity, stability and low toxicity.

The **net charge** on the dextran depends both on the attached fluorophore charge and on the capping reagent used to cap unreacted amines. Hence, capping yields typically neutral dextrans, quite neutral dextrans (i.e. with rhodamine and SR101 fluorophores that are zwitterionic) or anionic dextrans (with anionic fluorophores).

The **lysinated dextrans** are useful for applications that require the dextran tracer subsequently be treated with aldehyde fixatives for analysis. I.e. Lysine-fixable biotin-dextran (MW 10,000) is taken up by neuronal processes and transported bi-directionally. It infiltrates axon buttons in the anterograde direction and dendritic processes in the retrograde direction. Staining can be observed in fixed and sectioned tissue from two days to two weeks after biotin-dextran is injected into the brain or spinal cord. It can also be applied to cut nerve tracts. Biotinylated neurons are detected by either light or electron microscopy following incubation with avidin-horseradish peroxidase conjugate and the electron-dense substrate 3,3′-diaminobenzidine (DAB).

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Reference: Am J Physiol. 1990 Feb;258(2 Pt 1):C309-17. Lencer WI, Weyer P, Verkman AS, Ausiello DA, Brown D.FITC-dextran as a probe for endosome function and localization in kidney. / Dextran-Fluorescein

General properties of Biotin label

Biotin is an indirect label popularized thanks to is versatility for detection, purification, and amplification systems. It bind with very high affinity ot avidin related molecules.

General properties of Fluorescein dyes

Fluorescein is surely the most popular fluorochrome since the first ages of fluorescence biotechnologies. It has excitation/emission maxima of λabs./λem. 494 /519 nm.
Advantages include * Relatively high absorption, * Excellent fluorescence quantum yield, * Excitation maxima ~494 nm closely matches the 488 nm spectral line of the argon-ion laser (confocal laser scanning microscopy, flow cytometry), * well characterized fluorophore with low cost and large referencement.
Drawbacks inlcude: * Poor sensitivity in some conditions or un-accurate detection while new alterantive are proposed for superior performances (i.e. our great FluoProbes®488 and 505 dyes).

General properties of Rhodamine dyes

Rhodamines based dyes have supplemented fluorescein-based dyes, as they offer longer wavelengths emission maxima, opening opportunities for multicolor labeling.

Tetramethylrhodamine (**TMR**) is the most popular rhodamine dye, and has excitation/emission maxima of ~555/580 nm.
Its carboxilic acid form (TAMRA) remains prominent for oligonucleotide labeling (DNA sequencing), whille its isothiocyanate derivative (**TRITC**) rose a larger popularity, having bright red-orange fluorescent with similar excitation/emission maxima to TMR. Now, the absorption and emission in pH 8 buffer are red-shifted approximately 8 nm compared to MeOH, with EC ~10% lower. λabs./λem. : 543/571nm. The absorption spectrum of TMR/TRITC-labeled proteins is frequently dependent on the labeling location and on the degree of substitution, and may even show splitting into two absorption peaks at about 520 and 550 nm. Such limitation can be adressed using alternative dyes, such as our excellent **FluoProbes®547H** that has brighter and more stable fluorescence.

General properties of CY_{anine} dyes

CY_{anine}-structure based dyes were introduced to cover more uniformaly the light spectrum, offering additionally some advantages over former fluorochromes. Although many structures modifications were proposed, most cyanine dyes self aggregate in aqueous solution or show cis-trans isomerization that decrease the fluorescence.
$CY_{anine}5.5$ has maximum excitation/emission at 673/692nm with an exctinction coefficient of 250 000$M^{-1}cm^{-1}$
$CY_{anine}7$ has maximum excitation/emission at 747/774 nm with an exctinction coefficient of 250 000$M^{-1}cm^{-1}$

Applications

Size Exclusion & permeability

The defined molecular weights of dextran are useful to study exclusion properties:

- vascular networks and cell membranes
- intracellular communication through gap junctions
- artificial polymer matrices

Dextrans provide a excellent technique for studying the **microvascular permeability** of healthy and diseased tissues. The study can be performed in real time by intravital fluorescence microscopy. An added benefit of the technique is high sensitivity and concentrations down to 1ng/ml can be detected in tissue fluids.
The major application of TRITC-dextran is for characterising the permeability of semi-permeable membrane either synthetic or natural (organ tissues). This is enabled by using TRITC-dextrans of various molecular weights. For example a TRITC-dextran 150 000 will be retained in the blood vessels since the fraction excreted by the kidneys is very low.

Cell Lineage

Excellent retention properties and low toxicity of dextran are taken to good account in:

- Tracing cell lineage (cancerogenesis, embryogenesis,...)

Caged fluorophore - dextran can be injected into cells at early development stages and then later activated.

Neuronal Tracing

Dextran conjugates with molecular weights up to 70 000 daltons are widely used to trace neuronal projections. The 3 000 MW dextran conjugates penetrate peripheral neuronal processes better and diffuse faster than higher-

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MW dextrans. Labeled dextrans can function efficiently as anterograde or retrograde tracers, depending on the study method and tissue type used.

Endocytosis

Labeled dextran are useful to monitor the uptake and internal processing of exogenous materials to study:

- endocytosis,
- endosome fusion,
- cell membrane changes,
- vesicular morphology.

Fluid Dynamics

The diffusion of the dextran tracer can monitored using fluorescence recovery after photobleaching (FRAP) techniques, or by photoactivation of caged fluorophore-dextran conjugates, to study:

- macromolecular diffusion through cytoplasm,
- fluid flow velocity,
- liposome encapsulation
- vascular flow in whole animals.

Directions for use

Handling and Storage

Dextran conjugates are provided as lyophilized 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.

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 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 4°C for several weeks, with the addition of 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 .

Protocols

Protocols can be found in the literatures, differing greatly with each application, dextran net charge, degree of labeling and nature of the dye. Below are give 2 protocols examples.

General properties of FITC-dextran

FITC-dextran is supplied as a yellow powder which freely dissolves in water giving a yellow solution. The FITC-subtituents are stable at normal pH (4-10) at room temperature. Autoclaving may lead to some slight release of free fluorescein amine. Sterilisation is preferentially performed by sterile filtration. FITC-dextran has been shown to be stable *in vivo* and has excellent biocompatablity. Studies by gel chromatography have shown that the FITC-substituents are essentially distributed evenly over the dextran molecule.
Excitation is best performed at 490nm and fluorescence measured at 520nm. The intensity is however dependent on pH and measurements should be made under controlled conditions.

General properties of TRITC-dextran

TRITC-dextran is supplied as a red powder which dissolves in water giving a red solution. The higher molecular weight products appear to be somewhat slower to dissolve than the corresponding FITC-dextrans. Some heating may facilitate dissolution. The TRITC-substituents are stable at normal pH (4-10) at room temperature. Autoclaving may lead to some slight release of free aminorhodamine. Sterilisation is preferentially performed by sterile filtration. TRITC-dextran has been shown to be stable in vivo and has excellent biocompatablity.
Excitation is best performed at 550nm and fluorescence measured at 585nm. The intensity is however dependent on pH and measurements should be made under controlled conditions.

Fluoro-Gold User Guide

A 10% solution of Fluoro-Ruby is made by dissolving 10mg of Fluoro-Ruby dry powder in 100µl of distilled water. It is then delivered via intracranial stereotaxic injection using a 1µl Hamilton microsyringe to pressure inject the tracer. Injection volumes typically range from 0,02-0,1 µl nd are gradually injected over a 10-15 minute interval. The animal is allowed to recover and is sacrified typically 4-14 days later.

The animal is then perfused with neutral buffered formaldehyde (10% formalin or 4% paraformaldehyde in 0,1M neutral phosphate buffer). The brain is removed and postfixed for a least overnight in the same fixative solution plus 20% sucrose for cryo-protection. The brain is then cut on a freezing sliding microtone or cryostat into sections usually between 20 and 40 microns in thickness.

The tissue sections are mounted on gelatin coated slides and are air dried on a slide warmer at 50-60°C for at least one half hour. The dry slides are then transferred to xylene clearing solution for at least one minute and then coverslipped with DPX mounting media. The slides can then be examined under an epifluorescent microscope using a filter suitable for visualizing TRITC ($\lambda_{Ex/Em}$: 540/600nm).

Guidelines for use depends on applications. Furthermore differences in molecular weight of the dextran, net charge, degree of labeling and nature of the dye may affect the experiment. Please so refer to the literature.

References

Araki N, *et al.*, "A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages, *J Cell Biol*, **135**, 1249 (1996) Article
Bannai H, *et al.*, "Kinesin dependent, rapid, bi-directional transport of ER sub-compartment in dendrites of hippocampal neurons.", *J Cell Sci*, **117**, 163 (2004) Article
Botelho RJ, *et al.*, "Role of COPI in phagosome maturation.", *J Biol Chem*, **275**, 15717 (2000) Article
Fritzsch B.,"Fast axonal diffusion of 3000 molecular weight dextran amines.", *J Neurosci Methods*, **50**, 95 (1993) Abstract
Henley JR, *et al.*, "Dynamin-mediated internalization of caveolae.", *J Cell Biol*, **141**, 85 (1998) Article
Kaneko T, *et al.*, "Improved retrograde axonal transport and subsequent visualization of tetramethylrhodamine (TMR) - dextran amine by means of an acidic injection vehicle and antibodies against TMR.", *J Neurosci Methods*, **65**, 157 (1996) Abstract
Kempers R, *et al.*, "Plasmodesmata between Sieve Element and Companion Cell of Extrafascicular Stem Phloem of *Cucurbita maxima* Permit Passage of 3 kDa Fluorescent Probes." *Plant J*, **4**, 567 (1993) Abstract
Mallard F, *et al.*, "Direct pathway from early/recycling endosomes to the Golgi apparatus revealed through the study of shiga toxin B-fragment transport.", *J Cell Biol*, **143**, 973 (1998) Article
Olsson Y. *et al*., Vascular permeability in the nervous system, *Acta Neuropathol*., 33:45-50 (1975) [TRITC-Dextran]
Rutili G.and Arfors K.E., Passage across the capillary and blood lymph barrier, *Microvasc.res*., 6:260 (1973) [TRITC-Dextran]
Svensjö E., Thesis, Leakage of macromolecules in postcapillary venules, Acta Uppsaliensis,34, Almquist and Wiksell, Stockholm, 1978 [TRITC-Dextran]
Tageson C. *et al*., Passage of molecules through the wall of the gastro-intestinal tract, *Scand. J.Gastroenterol*.,13:519-524 (1978) [TRITC-Dextran]
Yoon Y, *et al.*, "A novel dynamin-like protein associates with cytoplasmic vesicles and tubules of the endoplasmic reticulum in mammalian cells.", *J Cell Biol*, **140**, 779 (1998) Article

Related products

- Hydroxystilbamidine (FluoroGold) FP-40766A
- Hydroxystilbamidine 4% Solution, FP-JW7290
- Fast Blue, FP-CM0240
- True Blue Chloride, FP-M1159A
- Nuclear Yellow, FP-M1477B
- Histochoice Mounting media, 41927A

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

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