Eosin-Y-lysine-dextran

CAS Nr.: Not registered

MW: approx. 500 kDa

Representative Mol. Structure: $(C_{47}H_{38}Br_4N_3O_{13})_x$-$(C_{13}H_{20}O_8N_2)_y$-$(C_{13}H_{20}O_8N_2)_z$

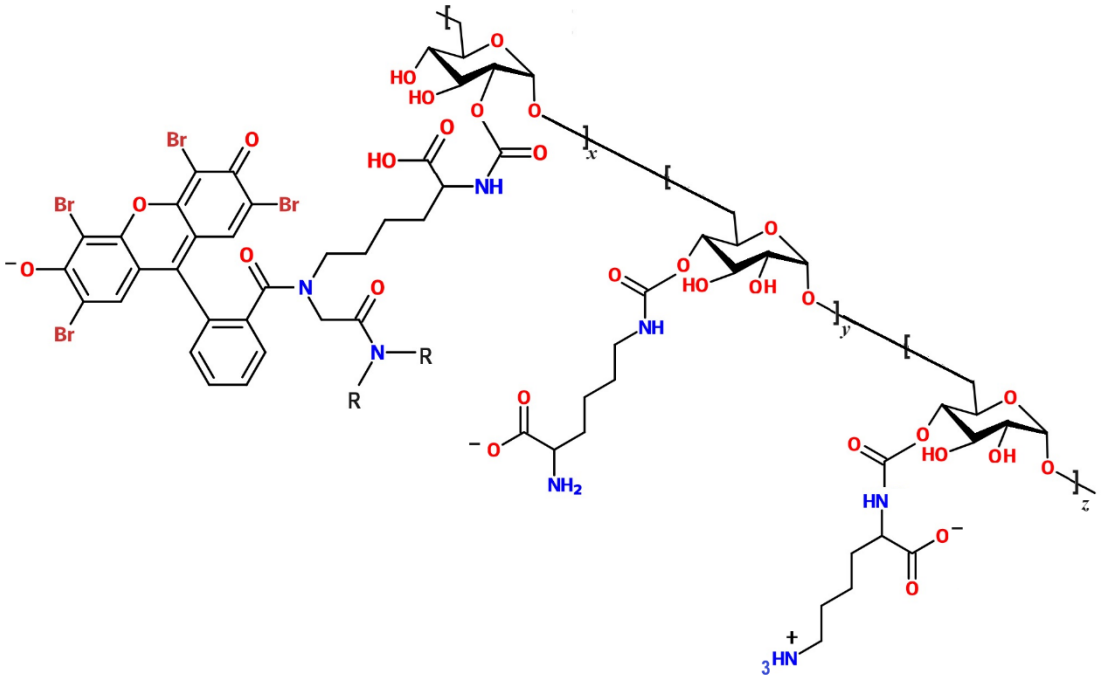

Fig. 1 Structure of Eosin-Y-lysine-dextran 500 kDa (ratio $x/(y+z)$ depends on the load of fluorescent labelling; R bulky substituent not displayed)

Brief description

Eosin-Y-lysine-dextran 500 (EYLD500) is a derivative of dextran that has been labeled with the dye Eosin Y (2-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl) benzoate; CAS No: 17372-87-1). Lysine dextran is marked using a special technique created by TdB Labs, which enables a significant level of functionalization (see Fig. 1). EYLD500 is delivered as a pink amorphous powder and exhibits bright orange fluorescence. It is soluble in water and non-protic polar organic solvents such as DMSO but remains insoluble in methanol and

ethanol. EYLD500 is part of a family of fixable fluorescent labeled lysine-dextran derivatives that has a range of potential applications in cell permeability studies, tissue and cell imaging, as a fluorescent nanocarrier for drug delivery applications, and as a pH indicator due to the pH-dependent fluorescence of the fluorophore (Eosin Y).

Regarding the substrate

Lysine-dextrans are synthesized from well-characterized dextran fractions derived from *Leuconostoc mesenteroides* functionalized with lysine. The dextran used is from Leuconostoc mesenteroides B-512F which is essentially a linear α-(1-6)-linked glucose chain with however a low percentage (2-5%) of α-(1-3) branches distributed along the chain. The dextran fractions used are carefully controlled by GPC, optical rotation, absorbance, and other control parameters.

A key property of lysine-dextran relies on the presence of primary amino group (part of the natural amino acid) which renders the fixation of lysine-dextran possible *via* reaction of the latter with an aldehyde such as glutaraldehyde forming a Schiff-base fixated on the surface of a cell or tissue. This property renders lysine-dextran an important scaffold for fixation. Stabilizing a fluorophore such as Eosin-Y on lysine dextran and applying the fixation strategy, efficient cell or tissue imaging can be achieved (see Fig. 2).

Synthesis and Structure

Labelling lysine dextran is achieved *via* a unique method developed at TdB Labs allowing for a high degree of functionalization (see Structure in Fig.1 and reaction pot in Fig. 4A). The method utilizes the amino groups of lysine dextran to achieve the formation of a linker that involves amide groups. The latter provides substantial spacing between the substrate and the fluorophore, and at the same time offers H-bonding opportunities (intramolecular and/or intermolecular, e.g., with solvent molecules or biomolecules e.g. proteins). The method is highly customizable, which allows for precise control over the labeling process. This way, different degrees of labeling can be achieved by making simple modifications.

After purification, the products are controlled for Mw, appearance, solubility, DS, pH, free lysine and free dye. The products are designated by the approximate molecular weights of the dextran fractions used. The actual molecular weight (approx. 500 kDa) is determined by a well-developed GPC method. This value is supplied with the Certificate of Analysis.

Solubility and Stability

EYLD500 is readily soluble at concentrations close to 100 mg/mL in water and 50 mg/mL in DMSO. To afford dissolution of EYLD500 at these concentrations, mild heating might be required (up to max. 60°C). The product is highly stable in its solid form at ambient conditions. If stored in a dark and dry place, EYLD500 powder has a guaranteed shelf life of 6 years. Solutions of EYLD500 are also stable, but it is recommended that they are stored at temperatures as low as -20°C for long-term use.

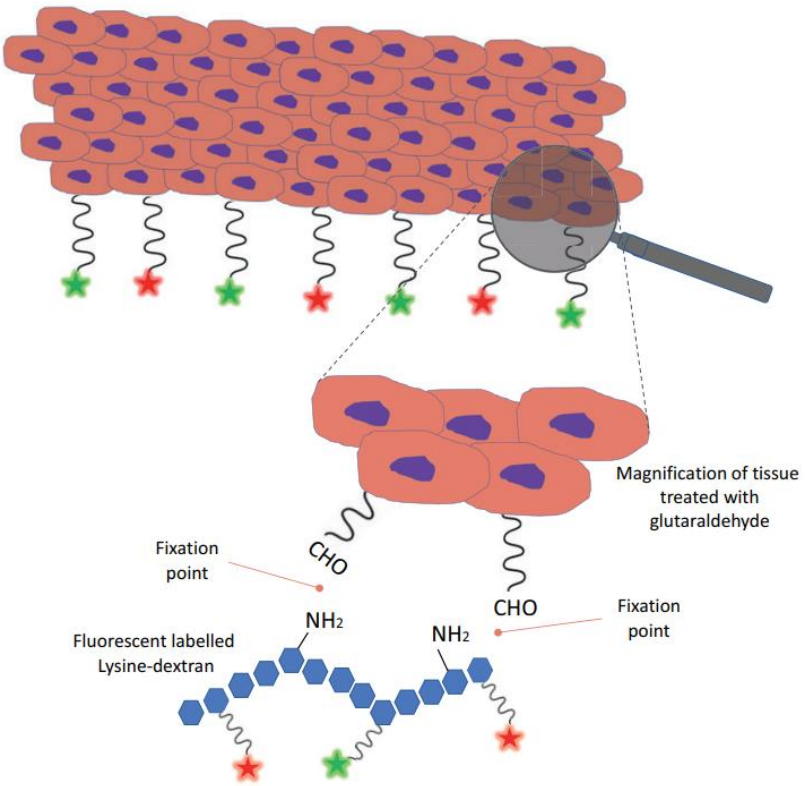

Fig. 2 Illustration of the fixation of labelled lysine dextran on tissue via reaction with paraformaldehyde or glutaraldehyde.

Fluorescence and optical properties

Due to the presence of Eosin Y, EYLD500 exhibits strong orange fluorescence (excitation max. at λ=530 nm and fluorescence max. at λ=548 nm in water; see Fig. 3). Its intense fluorescence is readily sensitive to pH changes (see section pH sensitivity, below). The fluorescence quantum yield for EYLD500 sample with a degree of fluorescent labelling of 0.002 mmol EY/g sample has been determined to be as high as Φ=0.91.

The emitted light at λ=548 nm falls within the green region of the electromagnetic spectrum. Nonetheless, solutions of EYLD500 in water appear as orange when irradiated at 365 nm using a black light source. In the solid state, EYLD500 has a pink color (Fig. 4B) which becomes intensely yellow when irradiated at 365 nm due to emission in the solid state (see Fig. 4C).

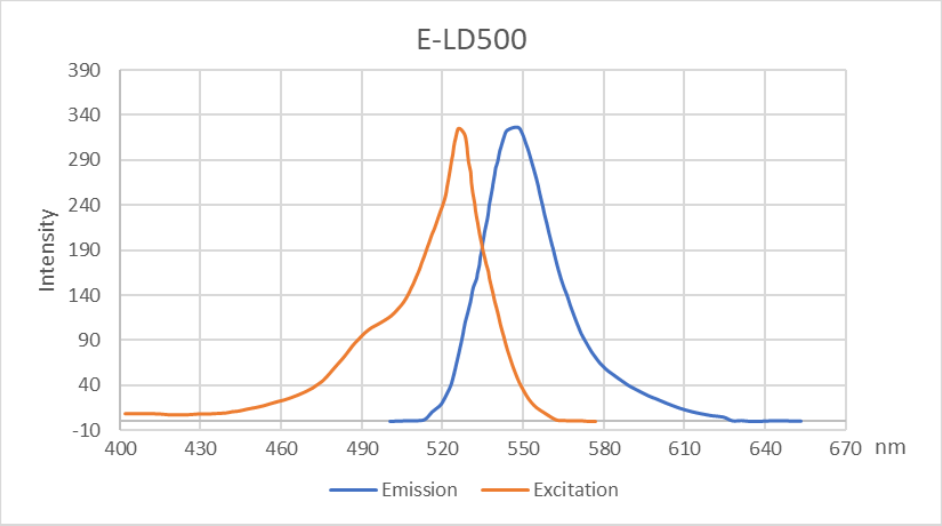

Fig. 3 Excitation and fluorescence spectra of EYLD500 recorded in borate buffer (pH=7.0)

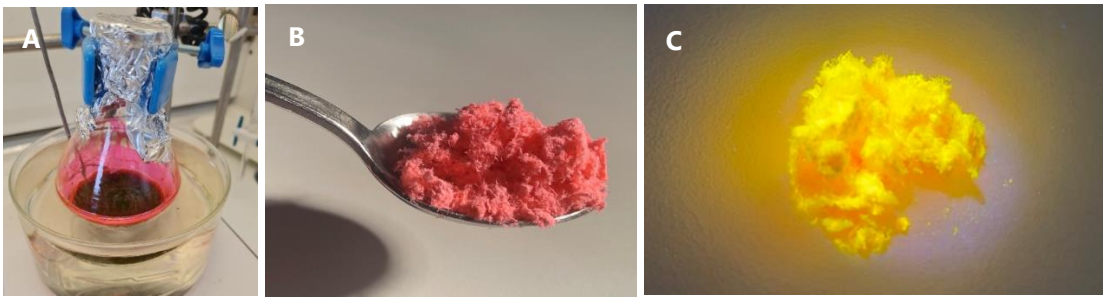

Fig. 4 A solution of EYLD500 in DMSO (A). A solid sample of EYLD500 under ambient light (B) and under UV light; 365 nm (C).

pH Sensitivity

It is noteworthy that EYLD500 is sensitive to pH due to the presence of Eosin Y, a tetrabrominated derivative of fluorescein that exhibits a similar dependency on pH like its parent compound. A decrease in the absorbance band of EYLD500 is observed when lowering the pH from 9 to 1. Additionally, a new band starts appearing at pH lower than 5, which is associated with the protonation of Eosin Y. At its protonated form, Eosin Y in solution appears as a nearly colorless/faint yellow compound. Similarly, the fluorescence of EYLD500 drops by a factor of approximately 7 upon the decrease of pH from 9 to 1. The optical behavior of EYLD500 against pH is reflected through the sigmoidal curve shown in Figure 5. This important feature of EYLD500 could be highly useful for the development of pH probes and generally environment-responsive biosensors.

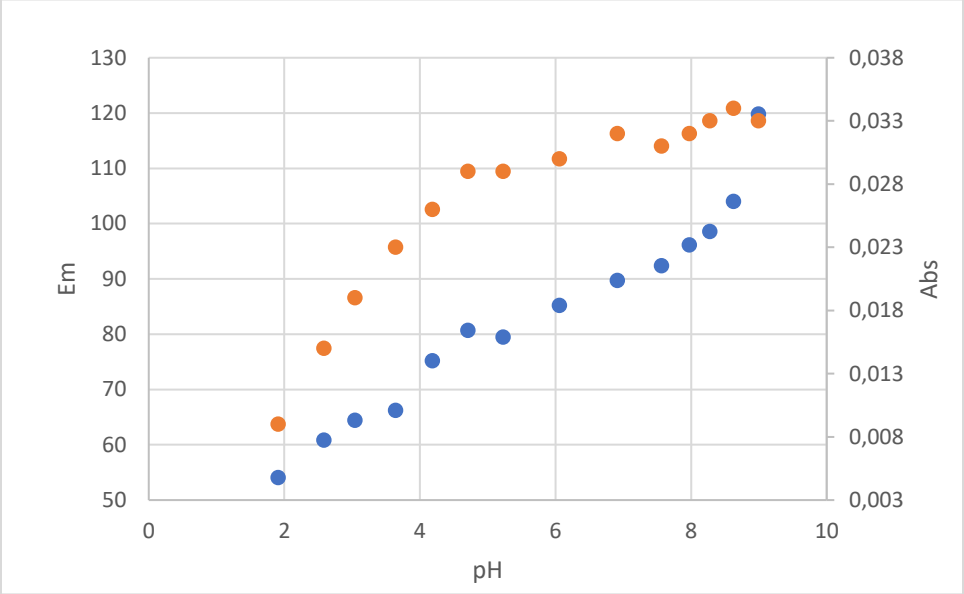

Fig. 5 Sigmoidal curves obtained from the results of titration experiments on EYLD500 indicating a high sensitivity to pH (absorbance: orange dots, emission: blue dots) in the range from 1 to 4 (Y-axis corresponds to the product of maximal absorbance or maximal emission intensity of EYLD500).