Eosin-Y-dextran

CAS Nr.: Not registered

Chemical Name: Dextran-(2,4,5,7-tetrabromo-6-oxido-3-oxo-3H-xanthen-9-yl)benzoate

MW: approx. 10 kDa

Representative Mol. Structure: $(C_{32}H_{33}Br_4N_2O_{10})_x$-$(C_6H_{13}O_5N)_y$

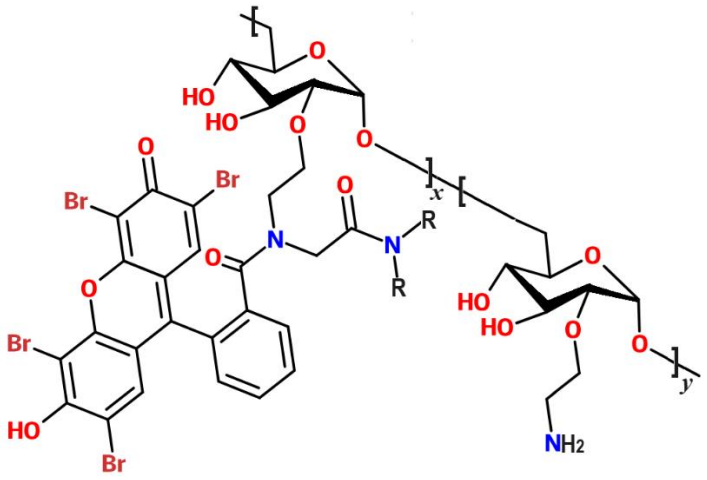

Fig. 1 Structure of Eosin-Y-dextran 10 kDa (ratio *x/y* depends on the load of fluorescent labelling; R bulky substituent not displayed)

Brief description

Eosin-Y-dextran 10 (EYD10) is a dextran-based derivative 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). Dextran is marked using a special technique created by TdB Labs, which enables a significant level of functionalization (see Structure in Fig.1). EYD10 is delivered as a deep red colored amorphous powder and exhibits marked fluorescence (orange). The product is readily soluble in water as well as non-protic polar organic solvents such as DMSO, however remains insoluble in methanol and ethanol. EYD10 has been designed to complement the family of fluorescent labelled dextran derivatives with a range of potential applications in cell permeability studies, as a fluorescent nanocarrier for drug delivery applications and research thereof, and as a pH indicator. Like all TdB Labs dextran derivatives, EYD10 is produced from selected dextran fractions. Dextran is derived from

the bacterium Leuconostoc mesenteroides B512F and consists of an α-D-(1 – 6) linear glucan with a low content (ca. 5%) of sidechains linked to the 3-carbon of glucose.

Synthesis and Structure

Labelling of 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. 3A). The method utilizes the amino groups of a previously functionalized dextran to create a linker that involves amide groups. This linker provides considerable spacing between the substrate and the fluorophore, while also offering H-bonding opportunities (intramolecular and/or intermolecular, e.g., with solvent molecules). 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.

Solubility and Stability

EYD10 is readily soluble at concentrations close to 100 mg/mL in water and 50 mg/mL in DMSO. To afford dissolution of EYD10 at the aforementioned 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, EYD10 powder has a guaranteed shelf life of 6 years. Solutions of EYD10 are also stable, but it is recommended that they are stored at temperatures as low as -20°C for long-term use.

Fluorescence and optical properties

Owing to the presence of Eosin Y, EYD10 exhibits strong orange fluorescence (excitation max. at λ=532 nm and fluorescence max. at λ=555 nm in water; see Fig. 2). Its intense fluorescence is readily sensitive to pH changes (see section pH sensitivity, below). The fluorescence quantum yield for EYD10 sample with a degree of fluorescent labelling of 0.003 mmol EY/g sample has been determined to be Φ=0.38.

The emitted light at λ=555 nm falls within the green region of the electromagnetic spectrum. Nonetheless, solutions of EYD10 in water appear as orange when irradiated at 365 nm using a black light source (see Fig. 3A). This is due to the intense color of the product itself. In the solid state, EYD has a dark ruby red color (Fig. 3B) which becomes intensely pink when irradiated at 365 nm due to emission in the solid state (see Fig. 3C).

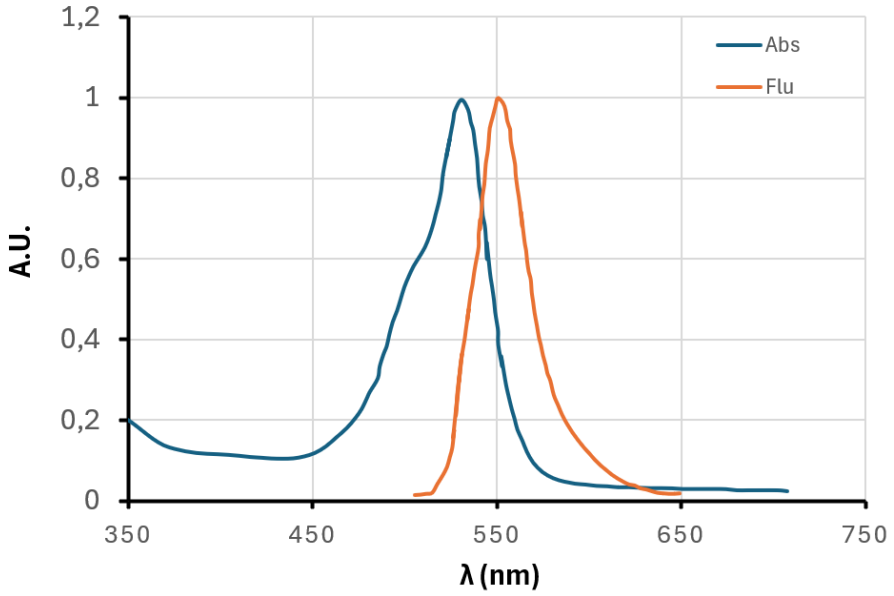

Fig. 2 Absorbance and fluorescence spectra of EYD10 recorded in borate buffer (pH=7.0)

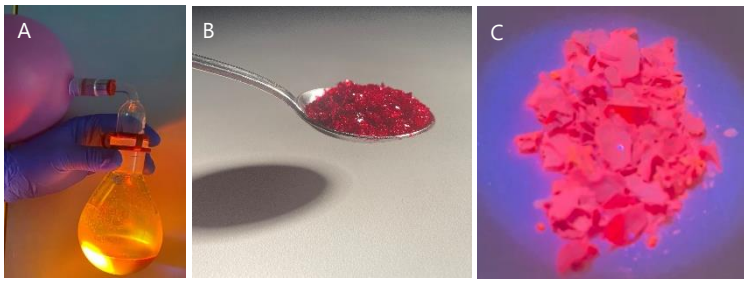

Fig. 3 A solution of EYD10 in DMSO under 365 nm light irradiation (A). A solid sample of EYD10 under ambient light (B) and under UV light; 365 nm (C).

pH Sensitivity

It is noteworthy that EYD10 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. As shown in Figure 4, a gradual decrease in the absorbance band of EYD10 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 EYD10 drops by a factor of approximately 7 upon the decrease of pH from 9 to 1. The optical behavior of EYD10 against pH is reflected through the sigmoidal curve shown in Figure 5.

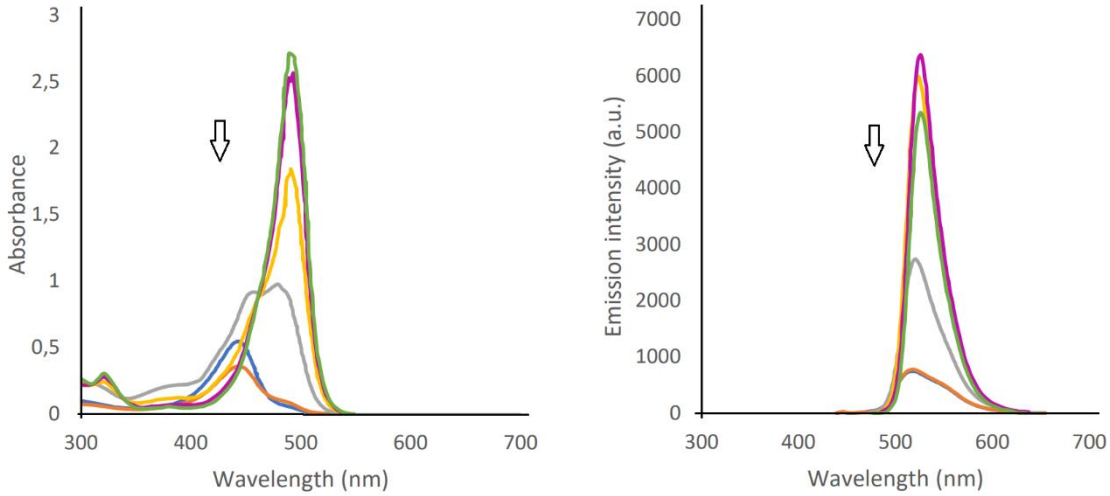

Fig. 4 Spectrophotometry experiments indicating the dependence on pH of absorbance (left) and emission (right) spectra of EYD10 recorded in water at 25°C. Arrows indicate the observed decrease of absorbance or emission intensity upon pH decrease from 9 to 1.

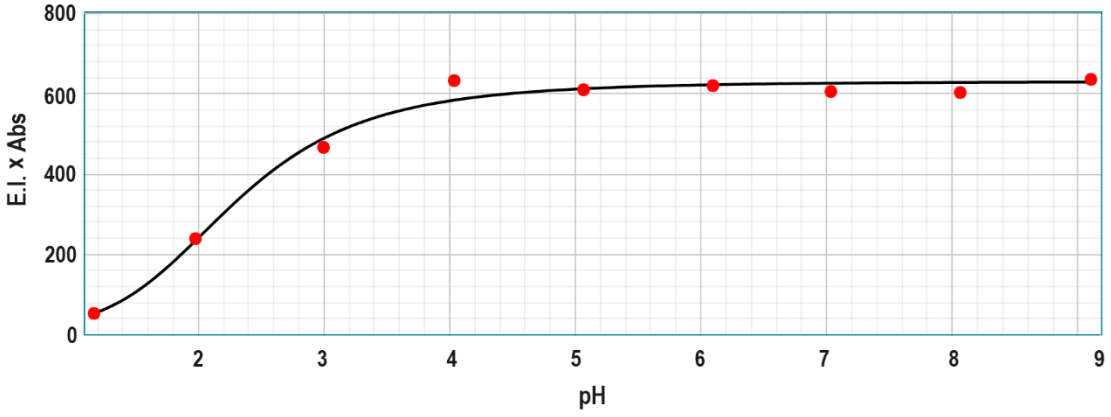

Fig. 5 Sigmoidal curve obtained from the results of the titration experiments on EYD10 indicating a high sensitivity to pH in the range from 1 to 4 (Y-axis corresponds to the product of maximal absorbance and maximal Emission Intensity of EYD10).

This important feature of EYD10 could be highly useful for the development of pH probes and generally environment-responsive biosensors and potentially diagnostic systems.