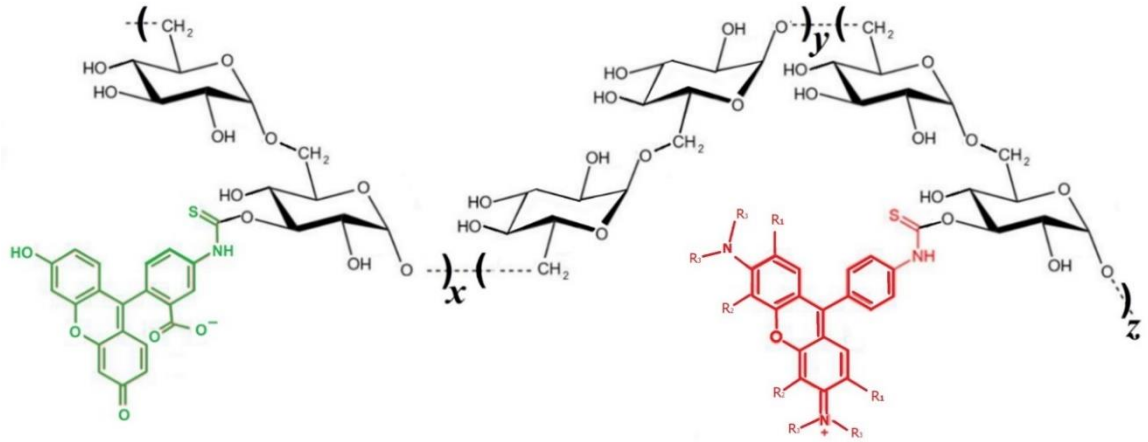
FITC-Antonia Red-dextran

CAS Nr.: N/A

Alternative Name: FARD

Structure:



x≈z<<y

Fig. 1 Structural representation of FITC-Antonia Red-dextran

Brief description

FITC-Antonia Red-dextran (FARD) is a dually labelled dextran derivative involving two fluorophores namely: FITC and Antonia Red (see Fig. 1). This derivative is currently produced at a molecular weight (MW) of 20 kDa and it is suitable for applications within intravital microscopy where accurate monitoring of pH in living cells or tissue is desired. FARD can operate at a wide range of pH spanning from 3.5 to 8.0.

Synthesis and Structure

FARD is synthesized from well-characterized dextran fractions derived from *Leuconostoc mesenteroides*. Dual labelling of dextran with FITC and AR is achieved via a well-optimized method allowing control of the FITC/AR labelling ratio. The fluorescent labelling ratio (FITC/AR) is assessed carefully via a combined method involving UV-Vis and fluorescence spectrophotometry.

Structurally, each of the fluorophores (FITC and AR) are bound to dextran through thiourethane linkages (Fig. 1). The degree of substitution (DS) lies in the range from 0.001 to 0.020 mol of dye per mole of glucose units for each of the fluorophores. The fluorescence intensity ratio of FITC/AR bound to dextran can vary between 1:1 and 1:3 and it is assessed spectrophotometrically at neutral pH.

The purification process (very similar to corresponding FITC- and AR-dextrans) leads to final FARD product with no traceable free-dye (i.e. non-bound dye or dye byproducts). Typically, the free-dye content in every QC-approved batch should not exceed 100 ppm (i.e. less than 100 µg of dye per gram of dry product). Free-dye content in FARD is assessed trough a novel and accurate chromatographic methodology developed at TdB Labs. After purification from non-bound dye, the products are controlled for MW, appearance, solubility, DS, and fluorescence. The actual molecular weight is determined by GPC. This value is supplied with the Certificate of Analysis.

Properties

The two fluorophores employed in FARD20 are well-studied and they exhibit bright and stable fluorescence which renders them suitable for a variety of applications in microscopy, fluorescence imaging, etc. FITC is a widely known fluorescent compound used for the fluorescent labelling of biologically relevant substrates e.g., proteins, polysaccharides etc. (see green labelled structure in Fig. 1). Antonia Red is TdB Labs' proprietary fluorescent dye (structure marked in red in Fig. 1). While FITC is fluorescein-based, AR is a julolidine-involving rhodamine. The first is widely known to respond to pH changes especially in the pH range between 3.5 and 8.0. On the other hand, AR is a product exhibiting a nearly unchanged fluorescence behavior in the above-mentioned pH-range (see Fig. 2 for details).

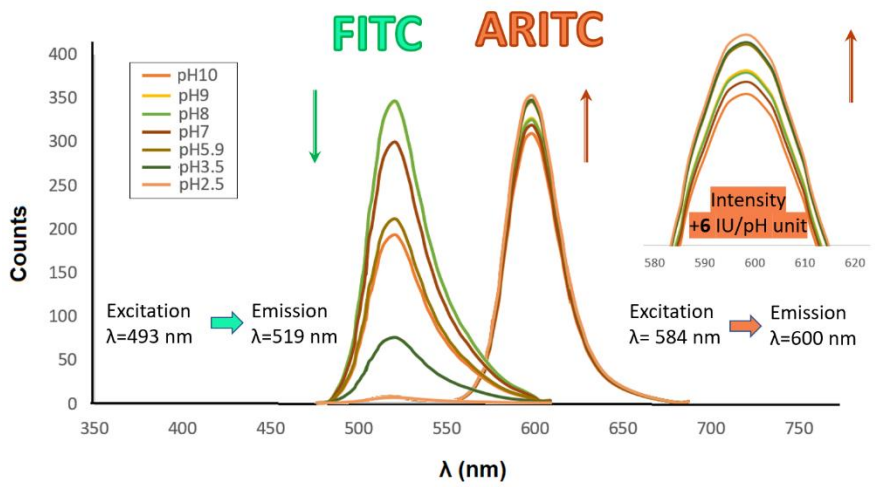


Fig. 2 UV-Vis spectra depicting the dependence of two visible features of FARD upon varying pH

As a result of the dual fluorescent labelling of dextran, FITC-Antonia Red-dextran exhibits fluorescence emission at λ_{fl}=517±5nm when photoexcited at λ_{ex}=493 nm (due to FITC) and at λ_{fl}=600±5nm when photoexcited at λ_{ex}=585±5nm nm (due to AR) (refer to Fig. 3 for color details).

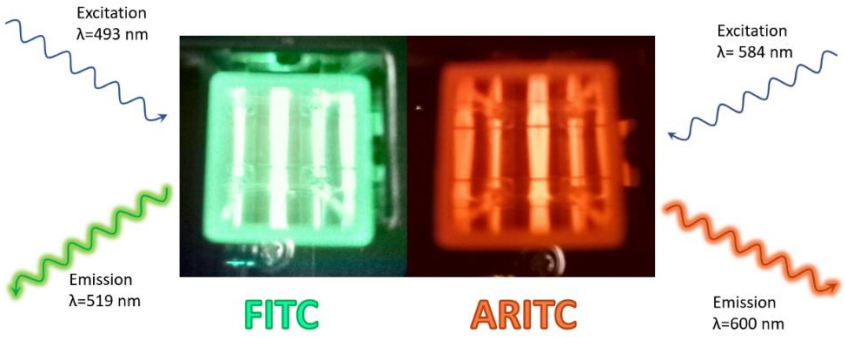


Fig. 3 Emitted color observed at different excitation wavelengths for a FARD20 product.

What renders this TdB Labs product unique is that the fluorescence (as well as the absorbance) of one of the fluorophores (FITC) is highly dependent on pH while the corresponding ones of the other fluorophore (AR) remains nearly unchanged between pH 2 and 10 (see Fig. 2). This allows for accurate determination of pH in living cells or tissues and thereby renders this product an invaluable tool for intravital microscopy.

The excellent performance of FARD is well illustrated through the sigmoidal curves of Fig. 4. When comparing pH dependence on the ratios of emission of FARD 20 to FITC-TRITC Dextran 500 (FTD500, another pH-probe provided by TdB Labs), the pH-dependence of both the products implies a very similar behavior of both the dyes. Yet, in case of FARD, a shift of the sigmoidal curve by around 0.5 of a pH unit to the lower is observed implying potential usability of FARD in applications involving slightly lower pH.

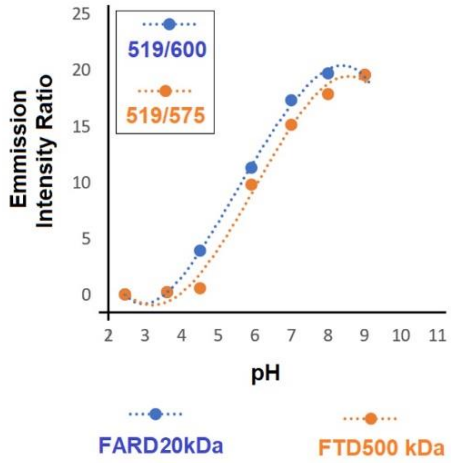


Fig. 4 pH-dependence on the ratios of emission of FARD20 and FITC-TRITC-Dextran 500 (FTD500).