FT-C3AIXA

Sensor 500 Green-PEG12 - SE ester

Fluorescent pH probe with improved solubility

Product Information

Name :	**Sensor 500 Green-PEG12 for Acidic pH - SE ester**
Catalog Number :	FP-C3AIXA, 1 mg
Solubility:	in DMSO
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 453 / 522 nm

Storage: –20°C >1 year Protect from light and moisture
Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Introduction

Sensor 500 Green-PEG12 SE was developed as an improved version of Sensor 500 Green SE, specifically to overcome the limited aqueous solubility of the original dye while retaining its excellent fluorescent and pH-responsive properties.

Both dyes exhibit identical excitation/emission spectra and the same pH response profile. However, the PEG12-modified version offers significantly enhanced solubility, facilitating conjugation workflows and improving overall assay performance.

Sensor 500 Green is a unique pH-sensitive fluorescent probe whose fluorescence increases as the environment becomes more acidic. In contrast to most conventional fluorescent dyes, which become brighter at higher pH, Sensor Green displays enhanced fluorescence under acidic conditions, making it particularly well suited for monitoring acidification processes in biological systems.

The dye remains essentially non-fluorescent at neutral extracellular pH but becomes brightly fluorescent upon exposure to acidic intracellular compartments. This pH-dependent activation minimizes background signal and eliminates the need for wash steps, resulting in improved assay sensitivity and reduced variability.

As a result, Sensor Green is an excellent tool for the specific detection and imaging of acidic organelles, including endosomes, lysosomes, and phagosomes, and is suitable for both fluorescence microscopy and flow cytometry applications.

In addition, Sensor Green possesses spectral characteristics similar to those of FITC, allowing it to be used with standard FITC filter sets and facilitating straightforward integration into existing imaging and analytical workflows.

Directions for use

Protein Stock Solution (Solution A)

Prepare the protein labeling solution by mixing 100 µL of reaction buffer (e.g., 1 M sodium carbonate or 1 M phosphate buffer, pH ~9.0) with 900 µL of the target protein solution (e.g., antibody; recommended protein concentration >2 mg/mL) to obtain 1 mL of labeling stock solution.
Important considerations

- The final pH of the protein solution (Solution A) should be 8.5 ± 0.5. If the pH is below 8.0, adjust it to pH 8.0-9.0 using either 1 M sodium bicarbonate or 1 M phosphate buffer (pH 9.0).

- For optimal results, the protein should be dissolved in 1X— phosphate-buffered saline (PBS), pH 7.2-7.4. Proteins prepared in Tris, glycine, or other amine-containing buffers must be dialyzed against PBS prior to labeling to remove free amines and ammonium salts (e.g., ammonium sulfate or ammonium acetate), which can compete with the conjugation reaction.

- High-purity protein preparations are recommended. Antibodies containing stabilizing proteins such as bovine serum albumin (BSA) or gelatin may show reduced labeling efficiency. Likewise, preservatives such as sodium azide or thimerosal may interfere with conjugation and should be removed by dialysis or desalting/spin-column purification before labeling.

- Labeling efficiency is strongly dependent on protein concentration. Concentrations below 2 mg/mL may result in substantially reduced conjugation yields. For best performance, a final protein concentration of 2-10 mg/mL is recommended.

Sensor 500 Green-PEG12 for Acidic pH - SE esterSolution (Solution B)

Reconstitute Sensor 500 Green-PEG12 SE by adding an appropriate volume of anhydrous DMSO to obtain a 10 mM stock solution. Mix thoroughly by pipetting or gentle vortexing until the dye is fully dissolved.
Important considerations

- Prepare the dye stock solution (Solution B) immediately before initiating the conjugation reaction whenever possible.

- For optimal labeling performance, use the freshly prepared dye solution promptly. Prolonged storage of the reconstituted dye may lead to hydrolysis of the NHS ester and a consequent reduction in conjugation efficiency.

- If necessary, Solution B may be stored at -20°C for up to two weeks, provided it is protected from light and moisture.

- To preserve dye activity, avoid repeated freezeâ€“thaw cycles by dispensing the stock solution into single-use aliquots before storage.

Example Labeling Protocol

This protocol has been optimized for the conjugation of Goat Anti-Mouse IgG with Sensor 500 Green-PEG12 SE. Depending on the specific characteristics of your protein, additional optimization may be required.

> Important: The optimal dye-to-protein molar ratio varies with both the protein and the fluorophore. Excessive labeling may impair protein activity or binding affinity, whereas insufficient labeling can reduce assay sensitivity and signal intensity.

Conjugation Reaction

1. As a starting point, use a 10:1 molar ratio of dye to protein. Add 5 µL of Sensor 500 Green-PEG12 SE stock solution (Solution B, 10 mM) to 95 µL of protein solution (Solution A) while gently mixing or vortexing.

   Example: For an antibody at 10 mg/mL with an approximate molecular weight of 200 kDa, the protein concentration is approximately 0.05 mM, resulting in a dye-to-protein molar ratio of about 10:1.

   > Optimization Tip: A 10:1 molar ratio is recommended as the initial condition. If necessary, optimize the degree of labeling by evaluating lower or higher dye-to-protein ratios such as 5:1, 15:1, and 20:1.

2. Incubate the reaction mixture for 30-60 minutes at room temperature, with continuous gentle shaking, rotation, or end-over-end mixing.

Purification of the Conjugate

The following procedure describes purification of the conjugated protein using a Sephadex G-25 desalting column to remove excess unconjugated dye.

1. Equilibrate and prepare the Sephadex G-25 column according to the manufacturer's instructions.

2. Carefully load the entire conjugation reaction mixture onto the top of the column bed.

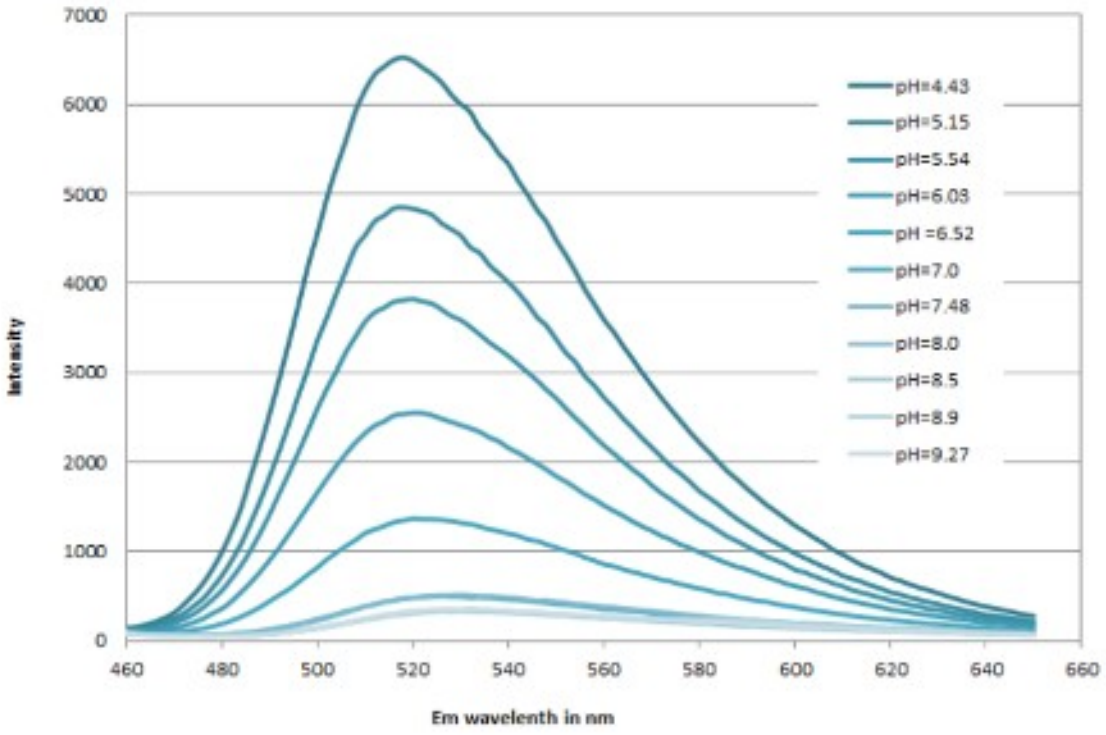
3. Once the sample has entered the resin and the liquid level reaches the top surface of the gel bed, begin elution with PBS (pH 7.2-7.4).

4. Continue eluting with PBS and collect fractions according to the column instructions. Identify and combine the fractions containing the purified Sensor 500 Green-labeled protein conjugate.

For immediate use: Dilute the purified conjugate in an appropriate staining or assay buffer and aliquot as needed.

For long-term storage: Store the dye-protein conjugate solution needs to be concentrated or freeze-dried.

The pH dependent Emission spectra of Sensor 500 Green-PEG12 SE



References

- **Wang Ke**, *et al.*, « Highly pH-Responsive Sensor Based on a EuIII Metal-Organic Framework with Efficient Recognition of Arginine and Lysine in Living Cells. », *Analytical chemistry*, 4992-4999 (2023)

Ordering information

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

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

Disclaimer : Materials from FluoProbes® are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use. FluoProbes® is not liable for any damage resulting from handling or contact with this product.

Rev.F09VB