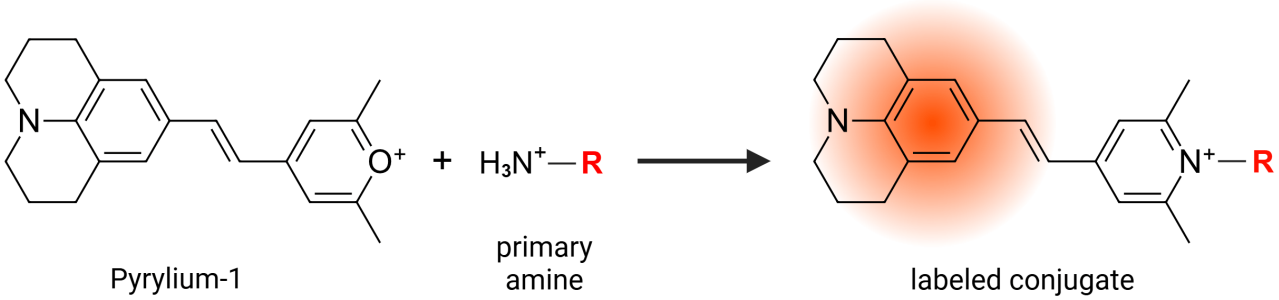
Protein Labeling with Pyrylium Dyes

Pyrylium dyes are fluorogenic dyes that are reactive with primary amines and form fluorescent products after conjugation with primary amino groups of peptides, proteins, and other biomolecules.



Pyrylium-1 | primary amine | labeled conjugate

In a free state, pyrylium dyes display a weak fluorescence, but their quantum yield increases significantly after conjugation with primary amines. Also, a short-wave spectral shift of the dye fluorescence is observed. The shift in excitation/emission spectra and the increase in quantum yield after conjugation contribute to a significant reduction in the background from the unbound dye. Unbound dye is also hydrolyzed during the labeling. All this allows labeling amine-containing molecules using a simple one-step incubation at room temperature without additional purification steps.

Pyrylium dye-labeled peptides and proteins are ready to use immediately after conjugation. Therefore, they can be used in a number of «no-wash» applications, such as SDS-protein gel electrophoresis, capillary electrophoresis, isoelectric focusing, etc. Pyrylium dyes are also used as a fluorescent label in receptor binding studies.

A typical protocol for labeling proteins with pyrylium dyes is provided below.

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Solution Preparation

1. *Dye Stock Solution*
   a. Dissolve 1 mg of dye in 100 µL of dimethylformamide.

      Important! Do not use amine-containing solutions or buffers as a solvent.

   b. The stock solution can be stored in the dark at 4 °C for 6 months.

2. *0.1 M Bicarbonate Buffer (pH 8.3)*
   a. Dissolve 4.2 g $NaHCO_3$ in 500 mL of bidistilled water.

   b. Adjust the buffer to pH 8.3 with 1 N NaOH.

3. *22 mM phosphate buffer (pH 7.2)*
   a. Dissolve 5.67 g $Na_2HPO_4 \times 12H_2O$ and 0.96 g $NaH_2PO_4 \times 2H_2O$ in 1 L of bidistilled water.

   b. Adjust the buffer to pH 7.2 with 1 N HCl.

Protein labeling

1. Dissolve 2 mg protein in 0.5 mL bicarbonate buffer (pH 8.3).
2. Add 5-10 µL of Pyrylium-1, -4, or -6 working solution dropwise to the protein solution.

   Add 10-20 µL of the Pyrylium-8 working solution dropwise to the protein solution.
3. Gently stir the reaction mixture at room temperature for the indicated time:
   - 30 min — for Pyrylium-1;
   - 1 h — for Pyrylium-4 and -6;
   - 2 h — for Pyrylium-8.

   Important! The dye is highly reactive in the pH range from 8.0 to 9.0.
4. When incubated in the basic solution, the reaction mixture will change color from blue (Pyrylium-1), violet (Pyrylium-4), or purple (Pyrylium-6) to yellow.
5. Unbound dye is hydrolyzed during the labeling procedure, so pyrylium dye-labeled peptides and proteins are ready for use immediately after conjugation.

Purification of the conjugated protein

For some applications, purification of the dye-conjugated protein may be required.

The labeled protein is purified by size exclusion chromatography using Sephadex G25 as the stationary phase and 22 mM phosphate buffer (pH 7.2) as the eluting agent. The red band indicates the labeled protein.

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