

Rhodamine 110 substrates for enzyme assays

Description

Rhodamine substrates are molecules consisting of one Rhodamine dye coupled to two identical peptides. In this conformation, the molecule is non-fluorescent.

Digestion of these substrates with proteases / peptidases results in a subsequent cleavage of the peptide from the dye. This leads to drastic conformational changes within the dye molecule, which turns the non-fluorescent molecule to a highly fluorescent one (see figure 1).

This change in fluorescence intensity makes this system valuable for determining enzyme activities. The spectral properties of Rhodamine 110 are similar to Fluorescein, but Rhodamine 110 is less sensitive to pH changes.

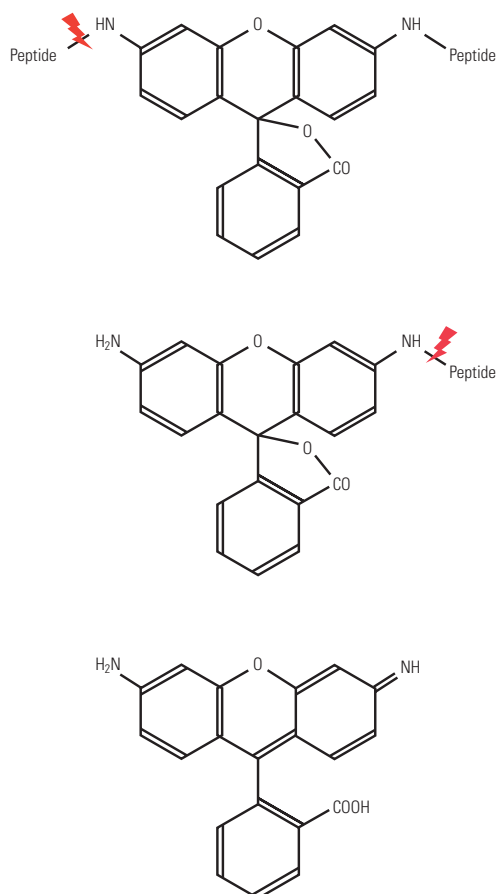
Advantages

- non-fluorescent substrate
- usually the substrate enters living cells easily
- Rhodamine110 resembles Fluorescein; but fluorescence is pH-independent
- linkage between peptide and dye is recognized as a peptidic bond by many enzymes

Applications

- protease activity assays in cuvettes
- protease activity assays in living cells (using fluorescence microscopy or flow cytometry)

Figure 1)
Generalmolecule structure



Abbreviation

Fluorescence intensity (relative)

Peptide₂-Rhodamine 110

0.0001 units



Peptide-Rhodamine110

0.1 units



Rhodamine110

1 unit

Product offering

Rhodamine Substrates are offered as follows:

amount	2 mg, 5 mg, 10 mg
length of peptide attached	1 - 5 amino acids
purity	> 95 %

The D2R-Substrate ((Asp)₂-Rh110) is available from stock. Other substrates are available on request.

Literature

1. Leytus S P, Patterson W L, Mangel W F (1982) New Class of Sensitive and Selective Fluorogenic Substrates for Serine Proteinases. J Am Chem Soc 104, pp. 7299-7306
2. Klingel S, Rothe G, Kellermann W, Valet G (1994) Flow cytometric determination of cysteine and serine proteinase activities in living cells with rhodamine 110 substrates. Methods Cell Biol 41, pp. 449-459
3. Ganesh S, Klingel S, Kahle H, Valet G (1995) Flow cytometric determination of aminopeptidase activities in viable cells using fluorogenic rhodamine 110 substrates. Cytometry 20, pp. 334-340
4. Hug H, Los M, Hirt W, Debatin K M (1999) Rhodamine 110-linked amino acids and peptides as substrates to measure caspase activity upon apoptosis induction in intact cells. Biochemistry 38(42), pp. 13906-11

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