Fluorescent Deoxyribonuclease I Conjugates

Quick Facts

Storage upon receipt:

- ≤–20°
- Protect from light

Ex/Em of Conjugates: See Table 1

Note: Do not vortex or shake solutions containing DNase I.

Introduction

Actin is one of the most ubiquitous and evolutionarily conserved structural proteins of muscle and nonmuscle cells and is generally present in two forms: G-actin (globular) and F-actin (fibrous). The transformation between these two forms relates to the need of the cell for structural rigidity or motion. By using selective fluorescent probes with very high affinity for G- or F-actin, one can obtain information on the localization of G- and F-actin in fixed cells.

DNase I has been used to detect and measure unpolymerized actin;[1–5] it binds to G-actin with an affinity of about 5×10^8 M^{-1}. Molecular Probes offers a broad selection of fluorescent conjugates of bovine pancreatic DNase I (Table 1) for selectively labeling monomeric G-actin. Phallotoxin conjugates, also available from Molecular Probes, are typically used for the staining of F-actin.

The influence of cytochalasins on actin structure in monocytes has been quantitated by flow cytometry using Texas Red® DNase I (D972) and BODIPY® FL phallacidin (B607) to stain the G-actin and F-actin pools, respectively.[6] Fluorescent DNase I has also been used in a model system to study the interactions of nucleotides, cations, and cytochalasin D with monomeric actin.[7]

Table 1. Fluorescent DNase I conjugates and their spectral characteristics.

Cat #	Conjugate	Abs *	Em *
D12371	Alexa Fluor® 488	495	519
D12372	Alexa Fluor® 594	590	617
D972	Texas Red®	597	615

* Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. Complete spectra for these dyes are available at our website, probes.invitrogen.com.

Materials

DNase I conjugates are supplied lyophilized in unit sizes of 5 mg. For long-term storage, reconstitute at 5 mg/mL (~161 µM) in one of the following buffers:

- phosphate-buffered saline, pH 7.4 (PBS) + 50% v/v glycerol
- 20 mM Tris, pH 7.6 + 50 mM NaCl + 1 mM DTT + 0.1 mg/mL BSA + 50% v/v glycerol

Glycerol at 50% in these solutions will maintain the liquid phase at ≤–20°C without affecting the stability of the enzyme conjugate. To avoid nonspecific binding, use only freshly prepared solutions or solutions stored at ≤–20°C in glycerol. Pancreatic DNase I is *unusually sensitive* to physical denaturation by shaking. Mixing should be done by gentle inversion. The lyophilized enzyme is stable for at least one year when stored as recommended, protected from light. Reconstituted enzyme can also be stored at 2–6°C, but activity may decrease when stored longer than one day at this temperature.

Properties

The molecular weight of the DNase conjugates is about 31,000 daltons. Peak excitation and emission wavelengths are shown in Table 1.

Staining G-Actin with Fluorescent DNase

1. Grow adherent cells on the surface of a coverslip.

2. Wash cells with PBS to remove excess medium.

3. Fix cells in 3.7% formaldehyde in PBS at room temperature for 10–15 minutes. Methanol disrupts the cytoskeletal structure or dye binding, which results in the absence of filament staining.

4. Wash three times with PBS.

5. Permeabilize cells in cold 100% acetone at –20°C for 5 minutes. Permeabilization can also be achieved by incubation with 0.1% Triton X-100 in PBS for 5 minutes.

6. Air-dry the samples or immediately rehydrate in PBS for 5–10 minutes.

7. Wash three times with PBS.

8. To the coverslip, add 200 µL of a 9 µg/mL (or 0.3 µM) solution of fluorescent DNase I in buffer. *Optional:* To simultaneously label F-actin, also add 1 unit (200 µL of a 0.165 µM solution) of a fluorescent phallotoxin. Stain for 15–20 minutes.

9. Wash three times with PBS.

10. Air-dry the samples. *Optional:* Add a drop of *SlowFade*® Gold or ProLong® Gold antifade reagent to reduce photobleaching. Mount and seal.

References

1. J Cell Sci 66, 39 (1984); **2.** Anal Biochem 135, 22 (1983); **3.** Exp Cell Res 147, 240 (1983); **4.** Eur J Biochem 104, 367 (1980); **5.** J Biol Chem 255, 5668 (1980); **6.** J Biol Chem 269, 3159 (1994); **7.** Eur J Biochem 182, 267 (1989).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
D12371	deoxyribonuclease I, Alexa Fluor® 488 conjugate	5 mg
D12372	deoxyribonuclease I, Alexa Fluor® 594 conjugate	5 mg
D972	deoxyribonuclease I, Texas Red® conjugate	5 mg
P36930	ProLong® Gold antifade reagent	10 mL
S36930	*SlowFade*® Gold antifade reagent	10 mL

Contact Information

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