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DQ™ BSAs: Self-Quenched BODIPY® Dye Conjugates of Bovine Serum Albumin

D-12050 DQ™ Green BSA *special packaging*
D-12051 DQ™ Red BSA *special packaging*

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Note: Avoid freeze-thaw cycles after reconstitutuing

Introduction

Molecular Probes' DQ™ Green BSA and DQ Red BSA are derivatives of bovine serum albumin (BSA) that are labeled to such a high degree with our BODIPY® dyes, BODIPY FL and BODIPY TR-X, respectively, that the dyes are strongly self-quenched. Proteolysis of the BSA conjugates can be monitored easily because digestion results in dequenching — the released protein fragments that contain isolated fluorophores are brightly fluorescent.

Highly substituted fluorescein conjugates of proteins have been used previously for the detection of proteolytic activity.[1,2] Conjugates of BODIPY dyes, however, are superior in this application. At comparable degrees of substitution, these conjugates exhibit substantially greater fluorophore quenching than fluorescein conjugates. By using BODIPY dyes in the synthesis of DQ Green BSA and DQ Red BSA, we have produced protease substrates that have extremely low background fluorescence and high signal-to-noise ratios upon digestion. In addition, the BODIPY fluorophores have the advantage over fluorescein in being insensitive to pH from pH 3–11 and having narrow excitation and emission spectral bandwidths. The pH insensitivity allows the direct detection of proteolytic activity in situations where the pH is unknown and cannot be controlled or where the pH is known to be low, for example within lysosomes and endosomes; the detection of fluorescein derivatives is greatly diminished below pH 8. The narrow spectral properties of DQ Green BSA and DQ Red BSA digestion products are advantageous in multicolor imaging applications.

The DQ Green and DQ Red BSAs are designed to be useful for the visualization of proteolytic activity in a variety of applications. Because of their extremely low background fluorescence and high sensitivity to digestion by various proteases, we anticipate that these substrates will be particularly valuable in the imaging of extracellular or intracellular proteolytic activity. For example, a prototype of DQ Green BSA was recently used to demonstrate the oscillatory proteolytic activity (alternating with oxidative activity) of migrating neutrophils. A neutrophil making its way through a gelatin matrix containing DQ Green BSA leaves a trail of bright, green-fluorescent bands, indicating pulses of protease activity.[3,4] (See photo at www.probes.com/servlets/photo?fileid=g000072). In another example, substrates similar to DQ Green BSA were reacted with anti-BSA antibody to form immune complexes and applied to human neutrophils, where endocytosis, via the Fc receptors, and subsequent digestion in phagolysosomes was monitored by flow cytometry.[5] DQ Green BSA and DQ Red BSA are experimental protease substrates that we offer to researchers who wish to develop ultrasensitive and creative strategies for the microdetection of protease activity within cells,[6,7] in gels,[8] on glass surfaces or on nitrocellulose membranes.

Materials

Contents, Storage and Handling

DQ Green BSA and DQ Red BSA are both provided lyophilized, conveniently packaged in sets of five vials, each containing 1 mg of the protein conjugate. In the lyophilized form, the DQ BSA products will remain stable for six months or longer, stored at -20°C, desiccated and protected from light.

Each 1 mg sample of DQ BSA has been lyophilized from 200–400 µL of phosphate-buffered saline (PBS), pH 7.2, and can be reconstituted to make a 1 mg/mL solution by dissolving the contents of the vial in 1.0 mL of a suitable pH 7.0–8.0 buffer, e.g. PBS. Sonicating the vial will help solubilize the product. Once reconstituted, the solutions may be stored for several weeks at 4°C, protected from light. We recommend adding sodium azide at 2 mM as a preservative.

Spectral Properties

Upon proteolytic digestion, DQ Green BSA releases fragments that have excitation and emission maxima of ~505 nm and ~515 nm, respectively. Upon digestion, DQ Red BSA releases fragments that have excitation and emission maxima of ~590 nm and ~620 nm.

References

1. Biotechniques 20, 286 (1996); **2.** Anal Biochem 176, 261 (1989); **3.** FASEB J 10, A1924 (1996); **4.** Biophys J 74, 90 (1998); **5.** J Leukoc Biol 62, 329 (1997); **6.** Eur J Cell Biol 75, 192 (1998); **7.** Proc Natl Acad Sci USA 96, 15056 (1999); **8.** Exp Cell Res 260, 292 (2000).

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

Cat #	Product Name	Unit Size
D-12050	DQ™ Green BSA *special packaging*	5 x 1 mg
D-12051	DQ™ Red BSA *special packaging*	5 x 1 mg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

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