Revised: 18–March–2001

DQ™ Ovalbumin (D-12053)

Quick Facts

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

Ex/Em of digestion product: 505/515 nm

Note: Avoid freeze-thaw cycles after reconstituting

Introduction

DQ™ ovalbumin is a self-quenched conjugate of ovalbumin that exhibits bright green fluorescence upon proteolytic degradation. This substrate, which is labeled with our pH insensitive BODIPY® FL dye, is designed especially for the study of antigen processing and presentation.[1,2]

Most immunogenic proteins require processing by antigen-presenting cells (APCs) and subsequent surface expression before they can be recognized by $CD4^+$ T-helper cells. Antigen processing involves protein internalization, denaturation, reduction and proteolysis, followed by association of the resulting peptides with MHC class II molecules and surface expression.[3] Although antigen processing and presentation have been extensively studied, the exact sequence and detailed pathways for generating antigenic peptides have yet to be elucidated.

Fluorescein-labeled bovine serum albumin (FITC-BSA) has been used as a fluorogenic protein antigen for studying the real-time kinetics of antigen processing in live macrophages by flow cytometry,[4] two-photon fluorescence lifetime imaging microscopy [5] and fluorescence polarization techniques.[6] The FITC-BSA used in these experiments was heavily labeled with fluorescein, such that the intact conjugate is relatively non fluorescent due to autoquenching. Upon denaturation and proteolysis, this type of FITC-BSA probe becomes highly fluorescent, making it useful for monitoring intracellular trafficking and processing of BSA within live macrophages.

As with FITC-BSA, FITC-ovalbumin has been used to study antigen uptake.[7] Molecular Probes has improved upon this idea with the introduction of DQ ovalbumin. One of the major drawbacks of fluorescein, and thus the FITC-BSA and FITC-ovalbumin probes, is the pH dependence of its fluorescence — the fluorescence intensity is greatly reduced at acidic pH. DQ ovalbumin is conjugated to our proprietary BODIPY FL dye, which exhibits bright, photostable and pH insensitive fluorescence in a pH range of 3–9. Another advantage is that intact DQ ovalbumin is more highly quenched than unprocessed FITC-BSA or FITC-ovalbumin, yielding a lower background signal. This high degree of quenching requires fewer fluorophores per protein molecule with the BODIPY FL dye than with fluorescein, which is important for preservation of the protein's antigenic epitopes. Furthermore, the BODIPY FL dye, which is typically visualized using standard fluorescein optical filters, has the ability to form excimers: If digested fragments of DQ ovalbumin accumulate in organelles at a high enough concentration, the BODIPY FL excimers can be visualized using a red light–sensitive longpass filter. Although we offer DQ Green BSA (D-12050), which is also a BODIPY FL conjugate, we believe that DQ ovalbumin will have greater utility for studying antigen processing and presentation since ovalbumin is internalized via the mannose receptor–mediated endocytosis pathway and is thus processed more efficiently than BSA.[8]

Materials

Contents

DQ ovalbumin is supplied lyophilized in five separate vials, each containing 1 mg of conjugate.

Storage and Handling

Upon receipt, this product should be stored desiccated at -20°C, protected from light. When stored properly, lyophilized DQ ovalbumin will remain stable for at least six months.

Each 1 mg sample of DQ ovalbumin has been lyophilized from 200–400 µL of phosphate-buffered saline (PBS). A 1 mg/mL solution can be prepared by dissolving the contents of one vial in 1 mL of PBS. Once reconstituted, the solutions may be stored for two weeks at 4°C, protected from light. We recommend adding sodium azide at 2 mM as a preservative.

Properties

Upon proteolytic digestion, DQ ovalbumin releases fragments that have fluorescence excitation and emission maxima of approximately 505 and 515 nm, respectively. In addition, when the digested fragments of DQ ovalbumin accumulate in organelles at high concentration, the BODIPY FL fluorophore may form excimers that can be visualized using a red light–sensitive longpass filter, e.g. filters for tetramethylrhodamine or Texas Red® dye.

References

1. J Immunol 165, 49 (2000); **2.** Proc Natl Acad Sci USA 96, 15056 (1999); **3.** Roitt, I.M., Brostoff, J. and Male, D.K., in *Immunology, Third Edition*, Mosby (1989) pp. 6.1–6.14; **4.** Biol Cell 87, 95 (1996); **5.** J Microsc 185, 339 (1997); **6.** Cytometry 28, 25 (1997); **7.** J Immunol 159, 2177 (1997); **8.** Exp Cell Res 215, 17 (1994).

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

Cat #	Product Name	Unit Size
D-12053	DQ™ ovalbumin *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.

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