Self-Quenched BODIPY FL Conjugate of BSA (Green)

03/15

Store at -20°C.

Cat. No.: 7932-2	**2 x 1 mg vials**
Cat. No.: 7932-10	**10 x 1 mg vials**

DESCRIPTION: Bovine serum albumin (BSA) is a common endocytic cargo, which typically ends up being degraded by proteases in the lysosome. Our Self-Quenched BODIPY FL Conjugate of BSA (Green) is a derivative of BSA. The BSA here is heavily labelled with fluorescence dye BODIPY FL. The high local concentration of the fluorophore on BSA causes prominent self-quenching of the dye conjugate. Proteolysis of the BSA releases peptide fragments that carry the fluorophores, thereby relieving the dye from self-quenching (de-quenching). Monitoring of the de-quenched fluorophore provides a reliable assay to monitor lysosome function, the lysosome cargo degradation, or assess proteolytic activity *in vitro*. This could be assayed using flow cytometry, fluorescence microscopy, confocal microscopy or a fluorescence microplate reader.

FORM: Lyophilized

FORMULATION: Lyophilized from approximately 10 mg/ml protein in PBS.

RECONSTITUTION: Reconstitute each vial in 100 µl water or PBS for a 10 mg/ml concentration. The suggested final concentration for cell culture applications is 10-20 µg/ml.

STORAGE CONDITIONS: The lyophilized product is stable for over 6 months at -20°C when stored desiccated and protected from light. Store the reconstituted aliquots at –20°C or –80°C.

APPLICATIONS/BENEFITS:

- The lysosome function/proteolytic activity can be detected with this conjugate by fluorescence microscopy, confocal microscopy, FACS analysis and fluorescence microplate reader.
- The extremely low background fluorescence of this conjugate and its high sensitivity to digestion by various proteases renders it particularly valuable in the imaging of extracellular or intracellular proteolytic activity.
- This conjugate has the advantage over fluorescein conjugates 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.

Figure 1

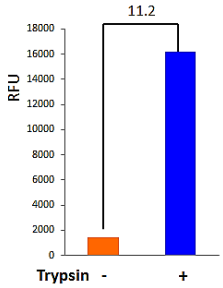

Figure 1. Fluorescence increase of De-quenched BODIPY FL BSA following it proteolysis: BODIPY FL BSA was de-salted into digestion buffer (Tris 50 mM, pH 8.0 and 2 mM $CaCl_2$). Then 100 µg BODIPY FL BSA (5 µg/µl) were digested with 5 µg trypsin (1µl, 5U/µl) at 37˚C for 3 hours. The digested BODIPY FL BSA was diluted to 10 µg/ml. The fluorescence intensities of the digested BODIPY FL BSA (+) and its non-digested counterpart (-) were measured at Ex/Em 490/520 nm with a 515 nm cutoff. Proteolysis of the self-quenched BODIPY FL BSA by trypsin resulted in de-quenching and increase in fluorescence of ~11.2 folds *in vitro*.

Figure 2

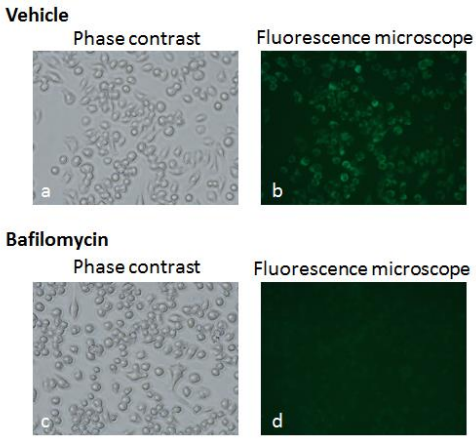

Figure 2. Lysosomal degradation of BODIPY FL BSA leads to increased fluorescence: Macrophages cultured in 12-well plates were first treated with vehicle or 100 nM bafilomycin (proton pump inhibitor) in growth medium (DMEM with 10% FBS) for 1 hr. Cells were then incubated with BODIPY FL BSA (16 µg/ml) in DMEM supplemented with 0.5% FBS with simultaneous incubation with either vehicle or 100 nM bafilomycin for another hr. The cells were then rinsed twice with ice-cold PBS supplemented with 2% FBS. The cells were visualized under phase contrast (a & c) and fluorescence microscope (b & d). We see increased fluorescence in the vehicle treated cells relative to the bafilomycin treated cells indicating the proteolytic digestion of the BODIPY BSA conjugate in the lysosome and its resultant de-quenching.

Figure 3

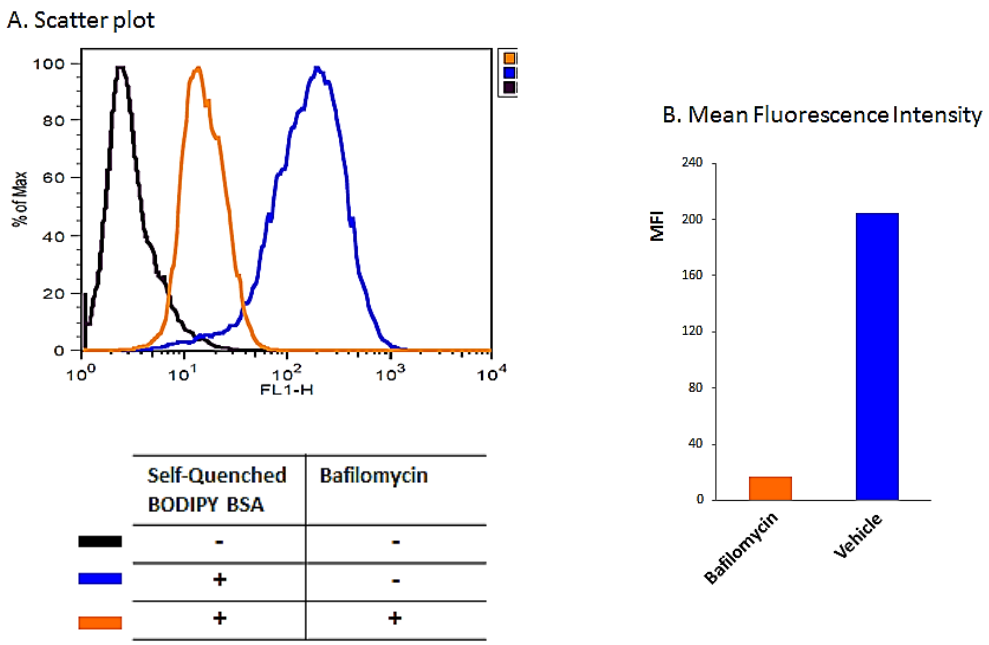

Figure 3. FACS analysis of BODIPY BSA in cells: Flow cytometry (BD FACSCalibur in FL1) was used to measure the fluorescence in macrophages incubated with Self-Quenched BODIPY BSA. Treatments were as detailed in the legend of Figure 2. A). Following exposure to Self-Quenched BODIPY BSA, vehicle-treated cells (Blue line) show much greater fluorescence intensity of BODIPY FL as compared to Bafilomycin-treated cells (Orange line). Untreated cells were used as negative control for the assay (Black line) B). Mean Fluorescence Intensity (MFI) for the quantitative representation of BODIPY FL intensity for the cells treated with either bafilomycin or vehicle shown in Figure 3A.

RELATED PRODUCTS:

- Biotin Quantitation Kit (**K811-100**)
- BSA-AGE (**2221-10**)
- Glucose AGE-BSA (**2223-10**)
- BSA (10% in H2O) (**2119-10**)
- Biotinylated Glucose AGE-BSA-II (**7930-250, 1000**)
- Biotinylated BSA (**7097, 7098, 7099**)
- Biotinylated BSA (Biotin-LC-BSA) (3 biotin/BSA) (**7097-5, 25**)
- Biotinylated BSA (Biotin-LC-BSA) (5 biotin/BSA) (**7098-5, 25**)
- Biotinylated BSA (Biotin-LC-BSA) (12 biotin/BSA) (**7099-5, 25**)
- Biotinylated AGE-BSA **(7929-250, 1000)**
- Biotinylated Glucose AGE-BSA-II **(7930-250, 1000)**

FOR RESEARCH USE ONLY! Not to be used on humans.