

Self-Quenched BODIPY Gelatin

05/15

Store at -20°C.

Cat. No.: 7935-30

30 µg

DESCRIPTION:

Gelatin consists of a heterogeneous mixture of high molecular weight proteins derived from collagen. These proteins can be cleaved by different cellular enzymes. Self-Quenched BODIPY conjugate of Gelatin (Type B) is an ideal fluorogenic substrate to detect protease activity of many such enzymes including collagenase, gelatinase, etc. BioVision's enhanced version of Self-Quenched BODIPY Gelatin can be used with very low amounts of enzymes for highly sensitive activity detection. For e.g., it can be used to detect as low as 6.5×10^{-5} U/ml of Clostridium Collagenase activity. Upon proteolytic digestion, the de-quenched BODIPY yields bright green fluorescence that can be assayed using a fluorescence microplate reader at Ex/Em: 490/520 nm with a 515 nm cutoff filter.

FORM: Liquid

FORMULATION: 3 mg/ml protein in PBS

STORAGE CONDITIONS: Stable for over 6 months at -20°C, protected from light.

APPLICATIONS/BENEFITS:

- Assay the activity of Collagenase, gelatinase and other proteases *in vitro*
- Zymography detection of the activity of Collagenase, gelatinase and other proteases

SUGGESTED PROTOCOL FOR ENZYMATIC ASSAYS:

- 1) Thaw the Self-Quenched BODIPY Gelatin at 37°C. Tap gently to mix well.
- 2) Prepare appropriate amount of the Self-Quenched BODIPY Gelatin by diluting 1:100 with PBS (Each reaction requires 10 µl [300 ng] of the diluted substrate).
- 3) Prepare 10X reaction buffer (0.5 M Tris, pH 7.6, 1.5 M NaCl and 50 mM CaCl₂)
- 4) Set up reaction as following:

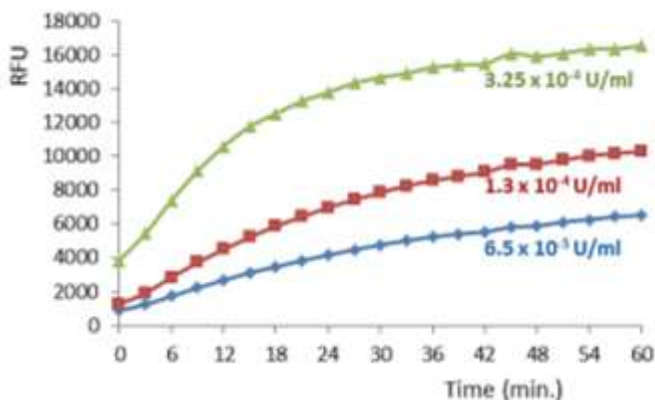
Reaction Buffer (10X):	10 µl
Diluted Self-Quenched BODIPY Gelatin:	10 µl
Enzyme/Sample:	x µl
dH ₂ O:	up to 100 µl

5) Measure the fluorescence at 37°C kinetically or as an endpoint assay (Ex/Em: 490/520 nm with a 515 nm cutoff filter).

Note: Plain Gelatin may be added to the reaction for kinetic assay.

For Gelatin Zymography, the amount of the Self-Quenched BODIPY Gelatin in the gel has to be experimentally determined.

Figure 1



Assay of Collagenase activity from *Clostridium histolyticum* using the Self-Quenched BODIPY Gelatin: Self-Quenched BODIPY Gelatin (300 ng per well) was used as substrate for the assay. Multiple concentrations of collagenase were incubated with the substrate, in the presence of 20 µg plain Gelatin, in a 100 µl reaction. The fluorescence (Ex/Em 490/520 nm, 515 nm cutoff filter) was monitored kinetically over 60 min. Background fluorescence, as determined in the reaction without the enzyme, was subtracted from each sample. As shown in Figure 1, BioVision's Self-Quenched BODIPY Gelatin measures Clostridium Collagenase activity at as low as 6.5×10^{-5} U/ml.

RELATED PRODUCTS:

- Self-Quenched BODIPY FL Conjugate of BSA (Green) (7932)
- Collagenase Activity Colorimetric Assay Kit (K792)

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