

Protease-Glo™ Assay

Interrogate Your Protease of Interest

- Sensitive protease detection
- P or P' compatible technology
- Bioluminescent readout



Promega

Benefits

- **Flexible:** For proteases with and without P' requirements
- **Avoid Fluorescent Background Problems:** Physical and chemical features of luminescence overcome compound screening problems due to fluorescence interference
- **Greater Sensitivity:** Ease and dynamic range of luminescence
- **Open Platform System:** Create your own protease substrates using standard molecular biology techniques, no chemical synthesis required
- **Interrogate Sequences:** Determine optimal protease recognition sequences or effects of amino acid substitutions
- **Web Application:** Make proper oligo design fast and easy; simply enter your amino acid sequence of interest

Description

The Protease-Glo™ Assay ^(a-f) is a novel method to detect and measure protease activities using a genetically engineered firefly luciferase and represents one example of the GloSensor™ platform technology (1). The assay uses a circularly permuted firefly luciferase, the GloSensor™-10F protein, with a protease recognition site as the protease substrate (Figure 1). This assay system allows rapid generation of protease substrates through molecular cloning and coupled transcription/translation cell-free expression, enabling the easy evaluation of protease function. Oligonucleotides encoding a protease recognition sequence are designed and cloned into the GloSensor™-10F gene located on a linearized vector. The GloSensor™ protein containing the protease site of interest is then synthesized in a cell-free protein expression system and subsequently used as a protease substrate. Cleavage of the protease recognition sequence leads to activation of the GloSensor™ protein and light emission (Figure 1). The level of luminescence correlates to protease activity (3). The Protease-Glo™ Assay has the advantage of a bioluminescent readout, which provides easy quantitation, high sensitivity and wide dynamic range (1–3).

Check out the Protease-Glo™ Assay Design Tool at: www.promega.com/techserv/tools to see how to design oligos for cloning your protease recognition site into the pGloSensor™-10F Linear Vector.

Applications:

- Compare related proteases (e.g., viral strains) against the same substrate
- Determine protease substrate specificity—length of essential sequence and which specific amino acids are essential
- Screen for protease inhibitors and determine IC₅₀ ranking

Design Concept for Protease-Glo™ Assay

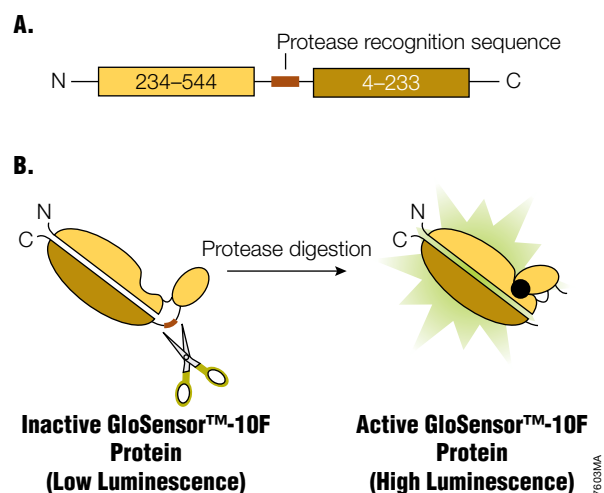


Figure 1. Modulation of firefly luciferase with a polypeptide linker between the N- and C-termini. Panel A. To generate the GloSensor™ protein, new N- and C-termini were created at amino acids 234 and 233, respectively. The protein-coding region of this circularly permuted firefly luciferase is carried on the pGloSensor™-10F Linear Vector. Panel B. Insertion of a protease recognition sequence between the native N- and C-termini of firefly luciferase greatly reduces luciferase activity while cleavage of the sequence by the cognate protease activates the luciferase enzyme.

Detection of Protease Activities

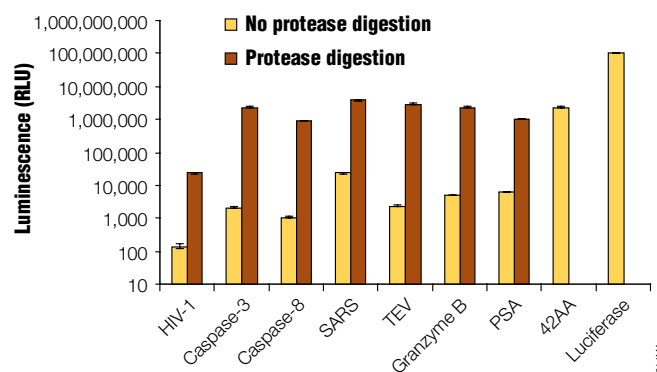


Figure 2. Examples of GloSensor™[protease site] activation by protease digestion. Seven different GloSensor™[protease site] proteins with protease recognition sequences for the indicated proteases were activated 150–1,250 fold upon proteolysis. A GloSensor™[42AA] protein containing a 42-amino acid sequence and the full-length firefly luciferase protein were created and used as controls. Additional experimental procedures can be found in the *Protease-Glo™ Assay Technical Manual #TM303*.

References

1. Fan, F. *et al.* (2008) Novel genetically encoded biosensors using firefly luciferase. *ACS Chem. Biol.* **3**, 346–51.
2. Fan, F. and Wood, K.V. (2007) Bioluminescent assays for high-throughput screening. *Assay Drug. Dev. Technol.* **5**, 127–136.
3. Wigdal, S. *et al.* (2008) A novel bioluminescent protease assay using engineered firefly luciferase. *Curr. Chem. Genomics* **1**, 94-106.

Ordering Information

Product	Size	Cat.#
Protease-Glo™ Assay	1 kit	G9451

Includes:

- 200µl Rapid Ligation Buffer
- 100 units T4 DNA Ligase
- 1.0µg pGloSensor™-10F Linear Vector
- 1ml Oligo Annealing Buffer
- 1.25ml Nuclease-Free Water
- 300µl TnT® SP6 High-Yield Wheat Germ Master Mix
- 10ml Bright-Glo™ Assay Buffer
- 1 vial Bright-Glo™ Luciferase Assay Substrate
- 15µg pGloSensor™-10F[TEV] Control Plasmid (150ng/µl)
- 1,000 U ProTEV Protease (10U/µl)
- 1ml ProTEV Buffer, 20X
- 250µl DTT, 100mM
- 1 Protocol

Storage Conditions: Store all components at –20°C, except the TnT® SP6 High-Yield Wheat Germ Master Mix, which must be stored at –70°C.

Available Separately

Product	Size	Cat.#
pGloSensor™-10F Linear Vector	1µg	G9461

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