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Product Information

HIV Protease Activity Detection Kit

Catalog Number **APPA014**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Cut-N-Glow™ is the first fully biological *in vivo* protease mapping tool that emits fluorescence. This assay is easily tailored via standard cloning techniques to detect specific proteases or protease inhibitors, or to map protease substrate preference *in vitro* or *in vivo*. Chemical synthesis is not required and there is no need for cofactors or co-substrates. Additionally, this assay only requires two reagents and both are proteins that can be easily obtained following overexpression of *E. coli.*

Proteases occur naturally in all organisms and are valuable tools in medical diagnostics, serving as initiators of cell signaling, as regulators of immune responses, and as agents of infectious disease. Therefore, mapping proteases in parasitic diseases and bacteria as well as assayable proteases associated with cancer could lead to the identification of shared structural similarities validating potential drug targets. The Cut-N-Glow approach involves the introduction of a structural distortion into one of the complementary fragments (GFP 11), through the use of a conditionally stable tether, which serves to constrain the N and C termini of GFP 11 closely in space, thereby, diminishing the mutual affinity of the two fragments and blocking protein self-assembly until the tether is cleaved. The distortion can be reversed upon site-specific proteolysis of the tether, resulting in GFP 11 fragment assembly with GFP 1-10, generating reconstituted, functional GFP. Depending on the tether, the chimeric GFP can serve as a substrate for proteases from the three major classes: serine, cysteine, and aspartic acid.

This kit is specific for the HIV Protease, a retroviral aspartyl protease that is critical to viral life-cycle.

Components

This kit contains sufficient reagents for one 96 well plate (96 assays).

Constrained Substrate Ready-to-use solution	1.0 mL
Detector (S1-10) Complementary GFP fragment Ready-to-use solution	20 mL
Positive Control Reagent, Ready-to-use solution	500 µL

Reagents and Equipment Required but Not Provided

- TE buffer, pH 2.0
- HIV protease (Cat. No. SRP2152)
- 96 well UV compatible microplate
- UV plate reader
- Humidified incubation system

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The components of this kit remain active for approximately 6 months when stored at –20 °C.

Depending on the particular usage requirements, it may be appropriate to re-aliquot reagents to smaller working volumes to avoid repeated freeze-thaw cycles or repeated pipetting from the same vial.

Procedure

In Vitro Assay

1. Equilibrate kit components to room temperature.
2. In a microplate well, mix 10 µL of Constrained Substrate with 10 µL of TE buffer, pH 2.0. Repeat for the number of wells as needed.
   Note: If assaying many samples, a 1:1 master mix of Constrained Substrate and TE buffer can be prepared. Pipette 20 µL of the master mix into each microplate well.
3. Add 0.5 µL of HIV protease to the reaction control well.
4. Add 1.0–10 µL of test sample to experimental reaction wells.
5. Incubate overnight at 37 °C in a humidified incubator.
6. Add 200 µL of Detector (S1-10) to test and controls wells and incubate plate at 37 °C for 6–16 hours.
7. Measure GFP fluorescence: $\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 525 nm.
   Note: Emission wavelengths can be ±25 nm

Results

Subtract the blank fluorescence values from the final fluorescence values of the sample(s) and the positive control. Perform appropriate statistical analysis, if applicable.

References

1. Callahan, B.P. et al., Protease Activation of Split Green Fluorescent Protein. ChemBioChem., **11**(16), 2259-63 (2010).

Cut-N-Glow is a trademark of Sandia Biotech, Inc.

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