

# IRDYE® NEAR-INFRARED FRET BASED ASSAYS

## IRDye HIV-1 Protease Assay Kit and $\beta$ -Secretase Assay Kit

The IRDye® HIV-1 Protease Assay Kit and IRDye  $\beta$ -Secretase Assay Kit (BACE-1) are near-infrared fluorescence energy transfer (NIR-FRET) based assays that introduce a new platform with enhanced sensitivity for protease activity detection. These assays utilize a novel, spectrally matched NIR dye FRET pair, IRDye 800CW and the new IRDye QC-1 Quencher, in conjunction with a LI-COR Odyssey® or Aeries® NIR imaging system for detection.

### Advantages

- Subnanomolar enzyme detection
- NIR detection dramatically reduces background, scattering and interference from other compounds
- Suitable for high throughput screening of enzyme activity
- Up to 28-fold (HIV-1) or 16-fold (BACE-1) fluorescence intensity enhancement upon digestion
- Inhibitor and drug candidate  $IC_{50}$  measurement
- Excellent water solubility

### Assay Descriptions

HIV-1 protease performs an essential step in the life cycle of HIV virus by cleaving *Gag* and *Gag-Pol* poly-proteins into the constituent proteins that make up infectious virus particles.<sup>1</sup> Anti-AIDS drugs targeting HIV-1 protease substantially suppress HIV viral replication and dramatically reduce mortality among HIV infected patients.<sup>2-3</sup>

Beta-Secretase (BACE-1) has been identified as a key enzyme that mediates a critical step in the formation of  $\beta$ -amyloid ( $A\beta_{40/42}$ ) by acting upon the  $\beta$ -amyloid precursor protein ( $\beta$ -APP)<sup>4</sup>. Deposits of  $\beta$ -amyloid form plaques in the brain that lead to damage and death of nerve cells. Plaques are one of the abnormal structures that characterize of Alzheimer's disease. Since discovery of the function of  $\beta$ -secretase, it has been established as a validated therapeutic target for Alzheimer's disease.

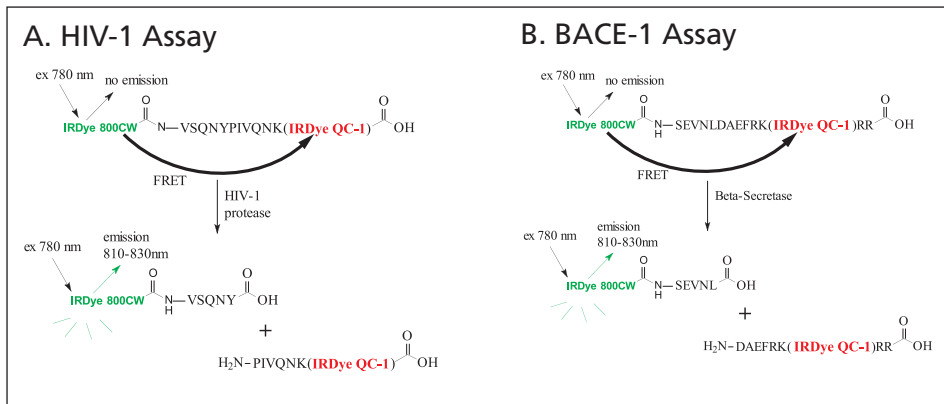


Figure 1. Principle of NIR-FRET based protease assays using the IRDye 800CW and IRDye QC-1 Quencher as a FRET pair. (A) for HIV-1 protease; (B) for BACE-1.

Figure 1 illustrates that both assays are based on highly quenched IRDye 800CW / IRDye QC-1 NIR-FRET peptide substrates. Cleavage of the peptide substrate separates the donor-quencher pair and restores fluorescence signal of the donor. Both assays utilize near-infrared excitation (780 nm) and near-infrared detection ( $820 \pm 10$  nm).

### Assay Performance

**Protease Detection.** Proteolytic cleavage of the peptide substrates in these assays increases with both incubation time and concentration (Figure 2).

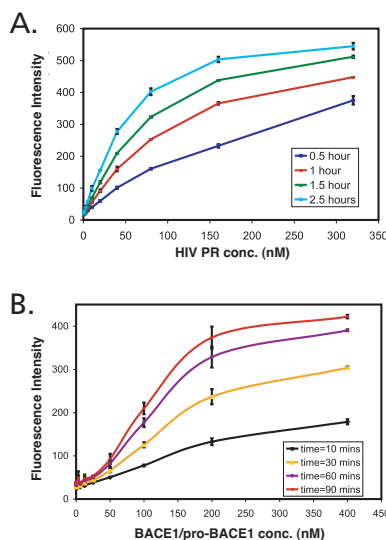


Figure 2. Effects of enzyme concentration and incubation time on the hydrolysis of HIV-1 protease substrate (A) and BACE-1 substrate (B).

**Protease Inhibition.** Figure 3 is an experimental result that illustrates the use of an IRDye HIV-1 Protease Assay Kit to measure the  $IC_{50}$  of a characterized HIV-1 protease inhibitor, Pepstatin A.

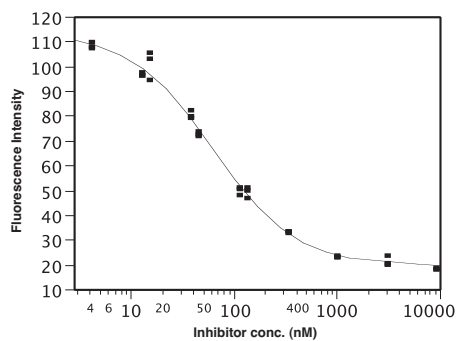
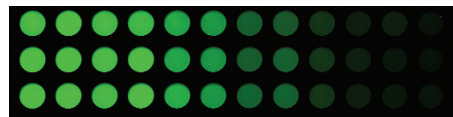


Figure 3. Inhibition of HIV-1 protease by a characterized inhibitor, Pepstatin A, in a 96-well plate. The serially diluted inhibitor solutions (10  $\mu$ L/well at final concentrations from 4.11 to 9000 nM) were mixed with HIV-1 protease (10  $\mu$ L/well at final concentration of 20 nM) for 15 minutes before adding substrate (20  $\mu$ L/well at final concentration of 1.0  $\mu$ M). After incubating at 37 °C for 1 hour, the reactions were stopped with 200  $\mu$ L/well of HIV-1 protease stop solution. Fluorescence intensity was measured on an Aeries Automated Infrared Imaging System.

**Low NIR Background.** Few compounds absorb or emit light in the detection region near 800 nm. Low background fluorescence interference at NIR wavelengths allows sensitive detection of enzyme activity, which otherwise would be difficult with visible fluorescence probes.

Figure 4 demonstrates that visible wavelength fluorescent compounds have no effect on assay signals. Similarly, color interference and light scattering are not significant for these NIR-FRET assays.

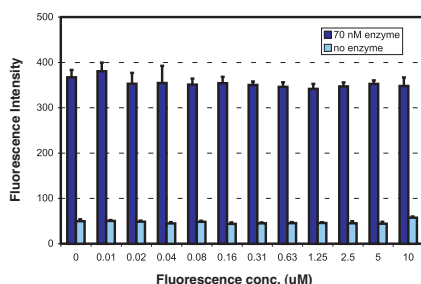


Figure 4. Fluorescein, a highly fluorescent compound, was introduced into the assay buffer to simulate the effect of background fluorescence in the visible spectrum. Concentrations up to 10  $\mu\text{M}$  had no effect on NIR background.

**Z'-Factor Analysis.** The HIV-1 protease assay shown in Figure 5 had 86% substrate conversion and its Z'-factor was calculated as 0.85 using the method of Zhang *et al*<sup>5</sup>. A similar assay at 35% substrate conversion had a Z'-factor of 0.73. The Z'-factor indicates whether assay conditions are optimized and if there is sufficient

dynamic range and low enough data variability to generate meaningful data. (Z'-factors of 1 indicate an ideal assay whereas assays with a Z'-factor less than 0.5 are qualitative). The assay in Figure 5 had a signal to background ratio of 14, a signal to noise ratio of 278, and a coefficient of variation of 4.68% across the wells containing the enzyme.

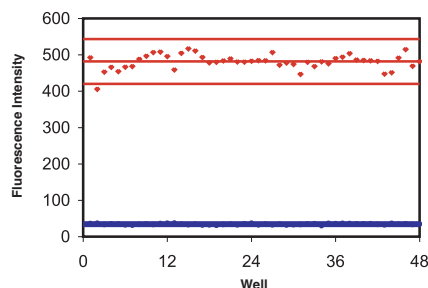


Figure 5. Z'-factor analysis of an HIV-1 protease assay in which the HIV-1 protease substrate (concentration 1  $\mu\text{M}$ ) was incubated with or without 80 nM HIV-1 protease.

**Excellent Water Solubility.** Both the IRDye 800CW near-infrared dye and QC-1 quencher have good water solubility and thus ensure that the dye-labeled peptide substrate is soluble in assay buffers without the need for an organic co-solvent.

**Effect of DMSO on Enzyme Activity.** For the IRDye HIV-1 Protease Assay, up to 10% DMSO can be added to improve library compound solubility. For the IRDye  $\beta$ -Secretase Assay, 2-3% DMSO can be added without affecting enzyme activity.

## Specifications

### IRDye 800CW

**Excitation Maximum:** 774 nm

**Emission Maximum:** 789 nm

## Ordering Information

**926-08588** IRDye HIV-1 Protease Assay Kit

**926-08589** IRDye  $\beta$ -Secretase Assay Kit

Each kit is sufficient for 100 assays (one 96-well plate) and includes the IRDye 800CW substrate quenched with IRDye QC-1 Quencher, assay buffer, enzymatic reaction stop solution, and protocol.

## Related Products

**929-70030** IRDye QC-1 Quencher (0.5 mg)

**929-70031** IRDye QC-1 Quencher (5.0 mg)

**929-70020** IRDye 800CW NHS Ester (0.5 mg)

**929-70021** IRDye 800CW NHS Ester (5.0 mg)

## References

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4647 Superior St. • P.O. Box 4000 • Lincoln, Nebraska 68504 USA  
 North America: 800-645-4267 • International: 402-467-0700 • FAX: 402-467-0819  
 LI-COR GmbH (Germany, Austria, Switzerland, Czech Republic, Hungary, and Slovakia):  
 +49 (0) 6172 17 17 771 • LI-COR UK Ltd.: +44 (0) 1223 422104 • [www.licor.com](http://www.licor.com)