A Sensitive Fluorimetric Assay for Detection of β-Secretase Activity Using a Novel FRET Peptide Substrate

Xudong Zhu, Xing Han, Manpreet Mann, Rich Meyer, Xiaohe Tong, Anita Hong and Vera Rakhmanova
AnaSpec Inc., 2149 O'Toole Ave, San Jose, CA 95131, USA

Introduction

Beta-secretase catalyzes a key step in the production of β-amyloid peptides seen accumulated in senile plagues of Alzheimer's disease (AD) brains. In order to facilitate high throughput screening of AD drug candidates, we have developed a new SensoLyte™ 520 β-secretase assay kit using a fluorescence resonance energy transfer (FRET) peptide, HiLyte Fluor™ 488-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys(QXL™ 520)-OH. The sequence of this FRET peptide is derived from the β-secretase cleavage site of β-amyloid precursor protein(APP) with Swedish mutation.[1] This mutation enhances the susceptibility of APP to β-secretase and results in an early onset of AD.

This assay has good sensitivity (0.03 mU/ml) and the signal-to-background ratio was over 10 after a 30-minute incubation. This homogeneous assay can be used to continuously monitor product formation. Assay was validated with known inhibitors and IC_{50} values were calculated.

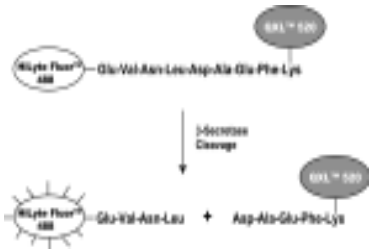

Figure 1. Proteolytic cleavage of HiLyte Fluor™488/QXL™520 FRET peptide by β-secretase. In the FRET peptide, the fluorescence of HiLyte Fluor™ 488 is quenched by QXL™ 520 until this peptide is cleaved into two separate fragments by β-secretase at the Leu-Asp bond. Upon cleavage, the fluorescence of HiLyte Fluor™ 488 is recovered, and can be continuously monitored at Ex/Em = 488 nm/520 nm.

FRET Substrate

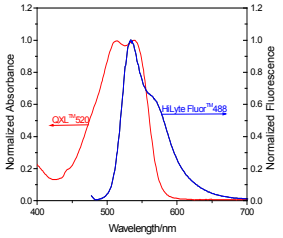

Figure 2. The absorption spectrum of QXL™520 overlaps with the emission spectrum of HiLyte Fluor™ 488. HiLyte Fluor™ 488 extinction coefficient is 92,400M^{-1} cm^{-1}.

Properties of HiLyte Fluor™488/QXL™520 pair:

- Ex/Em = 490 nm/520 nm for HiLyte Fluor™488
- Long wavelength fluorescence is less interfered by the short wavelength autofluorescence of drug candidates
- Better brightness of HiLyte Fluor™488
- HiLyte Fluor™ 488 is pH insensitive
- Hydrophilicity of QXL™ 520

Results

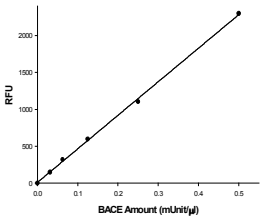

Figure 3. Sensitivity of the assay have been tested using serial dilution of enzyme. FRET substrate was incubated with the indicated amount of β-secretase* at 37°C and fluorescence was measured after 40 minutes using FlexStation 384II, Molecular Devices. Sensitivity of 520 β-secretase Assay was 0.03 mU/ml

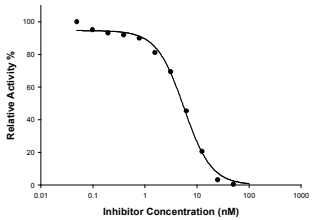

Figure 4. Inhibitor studies. To validate assay for inhibitor screening FRET substrate (20 mM) was incubated with enzyme in the presence of secretase inhibitor.** Kinetic readings were taken every 5 min for 30 min at 37°C (FlexStation 384II, Molecular Devices). The calculated IC_{50} was 5.62 nM.

*β-secretase enzyme (Cat# S5067, Sigma St. Louis, MO)
**β-secretase inhibitor KTEEISEVN-Sta-VAEF-NH2 was previously described in literature.[2]

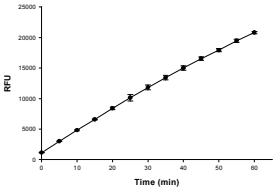

Figure 5. Assay kinetics. β-secretase (1 U) was incubated with 20 mM of the HiLyte Fluor™ 488/QXL™ 520 FRET substrate. Fluorescent signal was continuously monitored at Ex/Em=485±20 nm/ 528±20 nm for 60 min.

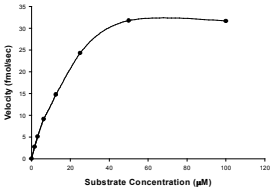

Figure 6A. Michaelis-Menton plot. Initial velocities (Vo) were calculated and plotted against substrate concentration, initial velocities expressed in fmol/sec.

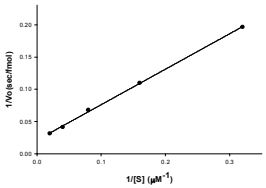

Figure 6B. Lineweaver-Burk plot. Lineweaver-Burk double-reciprocal plot for β -secretase with HiLyte Fluor™ 488/QXL™ 520 as substrate.

Conclusions

- We have developed a highly sensitive SensoLyte™ 520 β-secretase assay kit based on a HiLyte Fluor™ 488/QXL™ 520 FRET substrate.
- The longer excitation and emission wavelengths of HiLyte Fluor™488 minimize the interference from autofluorescence and absorbance of test compounds.
- This SensoLyte™ 520 β-secretase assay kit is capable of continuous, homogeneous monitoring of the enzymatic reaction.
- IC_{50} value for an inhibitor determined with SensoLyte™ 520 β-secretase assay kitwere consistent with published data.

References:

1. Mullan, M. et al. *Nat.Genet.* **1**, 345 (1992).
2. Sinha, S. et al. *Nature* **402**, 537 (1999).