



## **QTL Lightspeed™ $\beta$ -Secretase (BACE1, $\beta$ -site APP cleaving enzyme) Assay Kit**

### **Fluorescence Superquenching Assay System for the Quantitation of $\beta$ -Secretase in 96- or 384-Well Microplates**

#### **QTL BIOSYSTEMS**

**Cat. Nos.** QBS1 (1 x 384-Well), QBS10 (10 x 384-Well), QBS100 (100 x 384-Well)  
QBS4 (4 x 96-Well), QBS40 (40 x 96-Well), QBS400 (400 x 96-Well)  
1536-Well & 3456-Well Microplate formats: please inquire

#### **QTL Lit No. BACEK1.0**

### **I. INTRODUCTION**

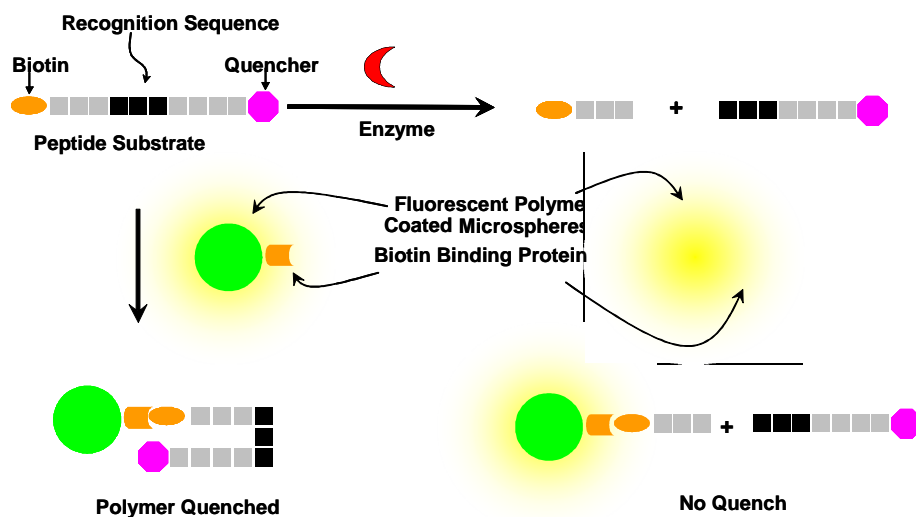
The  $\beta$ -Secretase (also identified as BACE1, Asp2 protease or Memapsin 2 or  $\beta$ -site APP-cleaving enzyme) is a type-I transmembrane aspartyl protease (1-3). The enzyme is involved in the deposition of  $\beta$ -amyloid, found in plaques of Alzheimer disease (1-3).  $\beta$ -Secretase has been identified as a key enzyme for studying both the physiology of Alzheimer's disease as well as to identify drug candidates that could alleviate, reverse or prevent the onset of Alzheimer's disease (1-3).

The QTL Lightspeed  $\beta$ -Secretase assay kit provides a fluorescent polymer superquenching-based assay format that is designed for the rapid and sensitive detection of enzyme activity and for screening potential  $\beta$ -secretase inhibitors. The kit is supplied with purified BACE1 enzyme and a peptide substrate based on the "Swedish" mutant. The kit contains enough reagents for 400 endpoint assays.

### **II. ASSAY THEORY**

The QTL Lightspeed technology is based upon fluorescent polymer superquenching (4-7) and is capable of providing up to several orders of magnitude greater sensitivity over comparable fluorescence resonance energy transfer (FRET)-based assays. The peptide substrate used in the QTL Lightspeed  $\beta$ -Secretase assay includes a quencher attached via the reactive peptide, which is recognized and cleaved by  $\beta$ -secretase to biotin. The QTL sensor is a fluorescent polymer that is co-located on a microsphere surface to biotin-binding protein. In the absence of enzyme in a sample, the peptide is intact and the quencher is brought in close proximity to the polymer to quench its fluorescence (background signal). When the peptide is recognized and cleaved by enzyme, the quencher and biotin groups are separated, and the quencher is unable to quench polymer fluorescence.  $\beta$ -Secretase activity is detected by an increase in fluorescence in the wells containing enzyme relative to wells without enzyme. A decrease in fluorescence will be observed in wells containing enzyme along with a  $\beta$ -Secretase inhibitor in comparison to

those that contain enzyme without inhibitor in proportion to the amount and potency of the inhibitor.



### III. KIT COMPONENTS

#### A. Materials Provided

Reagent	Composition	Amount	Catalog No.
$\beta$ -Secretase enzyme	100 ng/ $\mu$ l solution	3 $\mu$ g	S-001E
Peptide Substrate	Dissolved in DMSO	20 $\mu$ L	S-001P
Assay Buffer	100mM Sodium Acetate, pH 4.0	20mL	S-001AB
QTL Sensor	Microsphere suspension in buffer	20mL	S-001MS

#### B. Materials required but not provided

- Black or white microwell plates (96- or 384-wells)
- Pipettes
- Polypropylene vials
- Multiwell spectrofluorometer

### IV. STORAGE AND STABILITY

**1. Assay Buffer:** Catalog # S-001AB; 100 mM sodium acetate, pH 4.0  $\pm$  0.1; 20 mL. Store at 4°C when not in use and warm to  $\sim$ 25°C before use.

**2. Peptide Substrate:** Catalog # S-001P; Biotinylated  $\beta$ -Secretase recognition peptide with a dark quencher. Stock solution is 200  $\mu$ M in 100% DMSO; 20  $\mu$ L. Store protected from light at -80°C. Dilute to 10  $\mu$ M by performing a direct mix with deionized water. Store in 50  $\mu$ L or any



suitable aliquots inside 0.2 or 0.5mL snap-cap polypropylene tubes or similar, protected from light, at  $-80^{\circ}\text{C}$ . Do not subject either stock or diluted aliquots to multiple freeze-thaw cycles. “Working solutions” of concentration  $2.4\ \mu\text{M}$  should be prepared by dilution as needed using Assay Buffer (Catalog # S-001AB). Do not re-use substrate solution that was diluted in Assay Buffer after overnight storage.

**3. Enzyme:** Catalog # S-001E; BACE-1, Human recombinant  $\beta$ -Secretase;  $100\ \mu\text{g}/\text{mL}$  solution;  $30\ \mu\text{L}$  in  $100\ \text{mM}$  Sodium Acetate, pH 4.0, 10% glycerol. Store the enzyme at  $-80^{\circ}\text{C}$  in  $5\ \mu\text{L}$  aliquots inside 0.5 mL snap-cap polypropylene tubes or similar, at  $-80^{\circ}\text{C}$ . Do not subject the dissolved enzyme to multiple freeze-thaw cycles. Once removed from  $-80^{\circ}\text{C}$ , the enzyme solution may be diluted to desired final concentrations using Assay Buffer and must be used the same day. Store the diluted enzyme on ice or at  $4^{\circ}\text{C}$  until ready to use.

**4. QTL Sensor:** Catalog # S-001MS; 20 mL. Store at  $4^{\circ}\text{C}$  protected from light. Shake bottle vigorously for about 10 seconds before using. Some frothing will form, and this is normal. Transfer the required volume to a polypropylene container. Calculate required volume for each experiment on the basis of  $40\ \mu\text{L}$  Sensor to be delivered to each microplate well. Please note that the QTL Sensor will be slightly turbid or cloudy. This is normal.

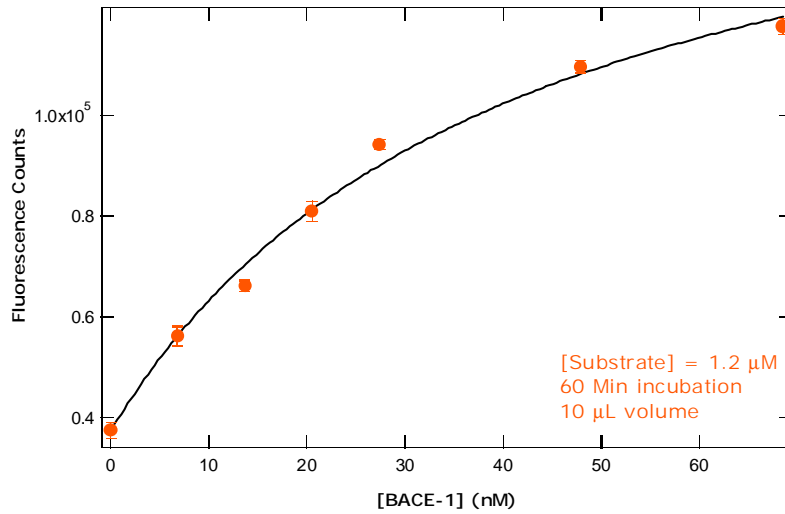
## V. QTL $\beta$ -SECRETASE ASSAY PROCEDURE:

### Step-by-Step: 96- and 384-well plate formats:

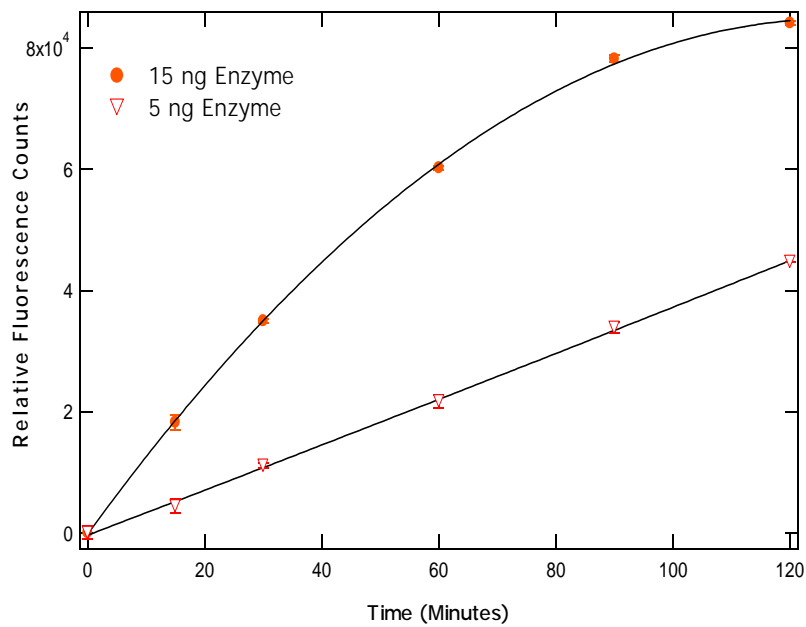
1. Dilute the enzyme solution in Assay Buffer to desired concentrations (We recommend  $1\ \text{ng} - 20\ \text{ng}$  enzyme in each reaction well corresponding to  $13.6\ \text{fmol} - 280\ \text{fmol}$  enzyme and equal to  $1.36\ \text{nM} - 28\ \text{nM}$  enzyme in each reaction). The enzyme may be added to each well in a volume of  $5\ \mu\text{L}$ .
2. Dilute the peptide substrate solution in Assay Buffer to a concentration of  $2.4\ \mu\text{M}$ . Add  $5\ \mu\text{L}$  of substrate solution to each well containing enzyme. Set up a control reaction by mixing together  $5\ \mu\text{L}$  of Assay Buffer and  $5\ \mu\text{L}$  peptide substrate solution.
3. We recommend a 30-second mixing step at this stage using the auto mix function of the microplate reader or using a plate shaker. **The total reaction volume in each well must be  $10\ \mu\text{L}$  for optimal performance.**
4. Allow the reaction to incubate at controlled room temperature (CRT;  $\sim 25^{\circ}\text{C}$ ) for 60 minutes. The reaction time may be reduced from 60 minutes if more enzyme is used or conversely lengthened to more than 60 minutes if less enzyme is present in the reaction mixture.
5. Terminate the enzymatic reaction by adding  $40\ \mu\text{L}$  of QTL Sensor to each well.
6. We recommend another 30-second mixing step at this stage using the auto mix function of the microplate reader or a plate shaker.
7. Check the fluorescence intensity of the sample and the control at emission  $\lambda_{\text{em}} = 576\ \text{nm}$  using excitation  $\lambda_{\text{ex}} = 440\ \text{nm}$ , and cut-off  $\lambda_{\text{co}} = 475\ \text{nm}$ . The difference in fluorescence intensity of the sample and the control is a measure of enzymatic activity.
8. To measure the fluorescence of the sensor alone without peptide substrate, mix together  $10\ \mu\text{L}$  of Assay Buffer and  $40\ \mu\text{L}$  of Sensor.

**VI. SAMPLE DATA**

**A.  $\beta$ -Secretase Concentration Curve**



**B. Time Course of the QTL Lightspeed  $\beta$ -Secretase Assay in 384-well plate format.**





#### **VII. SAFETY PRECAUTIONS:**

Normal precautions in handling laboratory reagents should be followed. The chemical, physical and toxicological properties of this kit have not, as yet, been thoroughly investigated. The use of gloves, protective eyewear and lab coat are recommended.

Purchaser will use purchased products solely for the purpose of conducting internal research at its' organization. This purchase provides the purchaser with a non-transferable right to use the product for research purposes only. Purchaser will not sell, transfer, disclose or otherwise provide access to the Purchased products by a Third Party. Purchaser agrees that Purchaser shall not have the right to authorize any third party use or sell and Products or derivatives of the Product unless a license has been executed between the Purchaser and QTL Biosystems specifically providing this. Purchaser will not (i) reformulate or create derivatives of the product, (ii) use the product for providing services to a third party, (iii) use the product in diagnostic processes, (iv) use the product in therapeutic processes, (v) use the product in a kit.

The performance of this product is warranted for a period of three months if the reagents are stored and handled according to recommendations.

QTL Lightspeed is a trademark of QTL Biosystems.

## VII. REFERENCES

1. Selkoe, D. J. Deciphering the genesis and fate of amyloid beta-protein yields novel therapies for Alzheimer disease. *J. Clin. Invest.* 110: 1375-1381, **2002**.
2. Citron, M. Human  $\beta$ -secretase and Alzheimer's disease. *Expert Opinion on Therapeutic Targets* 5: 341-348, **2001**.
3. Schmidt, B. Aspartic proteases involved in Alzheimer's disease. *ChemBioChem* 4: 366-378, **2003**.
4. Chen, L., McBranch, D.W., Wang, H.-L., Helgeson, R., Wudl, F. & Whitten, D.G. Highly sensitive biological and chemical sensors based on reversible fluorescence quenching in a conjugated polymer. *Proc. Natl. Acad. Sci.* 96: 12287-12292, **1999**.
5. Jones, R.M., Bergstedt, T.S., McBranch, D.W. & Whitten, D. Tuning of superquenching in layered and mixed fluorescent polyelectrolytes. *J. Am. Chem. Soc.* 123: 6726-6727, **2001**.
6. Lu, L., Helgeson, R., Jones, R.M., McBranch, D.W. & Whitten, D. Superquenching in cyanine pendant poly(L-lysine) dyes: dependence on molecular weight, solvent, and aggregation. *J. Am. Chem. Soc.* 124: 483-488, **2002**.
7. Kushon, S.A., Ley, K.D., Bradford, K., Jones, R.M., McBranch, D. & Whitten, D. Detection of DNA hybridization *via* fluorescent polymer superquenching. *Langmuir* 18: 7245-7249, **2002**.