

Drug Discovery FAQ's

What does β -secretase do and why is it important?

The accumulation of the 40- to 42-residue β -amyloid peptide ($A\beta$) in the brain is a key event in the pathogenesis of Alzheimer's disease. $A\beta$ is generated in vivo through proteolytic cleavage of the membrane-anchored β -amyloid precursor protein (APP) by β - and γ -secretase. The γ -secretase activity, which cleaves APP within its transmembrane domain, is likely mediated by the transmembrane protein presenilin 1. The β -secretase cleaves APP on the luminal side of the membrane and its activity is the rate-limiting step of $A\beta$ production in vivo. Both proteases are potential targets for inhibitor drugs against AD.

What is the theory behind the β -secretase assay kit?

The QTL Lightspeed technology is based upon fluorescent polymer superquenching 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 β -Secretase assay includes a quencher attached via the reactive peptide, which is recognized and cleaved by β -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. 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. β -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 β -Secretase inhibitor in comparison to those that contain enzyme without inhibitor in proportion to the amount and potency of the inhibitor.

What are the applications of the assay?

The QTL Lightspeed β -Secretase assay kit provides a fluorescence superquenching-based assay format that is designed for the rapid and sensitive detection of enzyme activity and for screening potential β -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.

Is the β -secretase assay kit ready to use out of the box or will I have to optimize the assay to suit my conditions?

The kit is designed to perform its best in 96-well and 384-well plates without any optimization and is ideal for high throughput screening. The assay speed can be modulated as desired by changing the amount of enzyme used in the assay. We recommend the use of 5 nanograms of enzyme in a 60-minute assay to achieve 20% conversion of substrate.

Will the performance of the assay change if I alter the conditions?

Any change made to the recommended assay procedures is likely to affect the assay performance. If you have any special requirements for your assay, please contact support@qtlbio.com to discuss with our technical representatives.

What are the assay's kit components?

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

What instruments are required to read the assay?

The QTL Lightspeed protease assay can be used in virtually all commercially available fluorescence spectrometers and multi-well plate readers. The excitation (blue) and detection (green) wavelengths are compatible with all common fluorimeters. QTL technical personnel can assist with configuring the settings to optimize performance of the QTL Lightspeed assay.

What will be the estimated cost per test kit?

Please contact sales for pricing inquiries. Contact information is on the web site under ordering information.

What are the materials that QTL Assay end-users need to supply?

In most situations, the end user will be required to supply a light-measuring instrument such as a fluorimeter, spectrophotometer or a microplate based detector and of course their samples containing the target of interest. If a field application is desired, QTL Biosystems could optionally supply the QTL Biosensor, a hand-held detector

Can the assay be optimized for Speed/Sensitivity?

The assay has been formatted to provide maximal sensitivity in a reasonable period of time (60 minutes). If a faster assay or greater signal is desired, the amount of enzyme used should be increased in the assay.

What well formats is the assay capable of using?

The assay has been formatted to work on 96-well or 384-well plates. The assay can also be performed in a miniaturized format. For more information, please contact QTL technical service.

Can I use black or clear plates?

The QTL Lightspeed β -secretase assay works equally well on white, black or clear plates.

What types of assay volumes are encountered in QTL assays?

The assay volume depends upon the unique applications of the QTL assay end-user. QTL assays could be performed in reaction volumes ranging from 20 μ l to 200 μ l. QTL assays could also be modified to nanoliter volumes in order to adapt to high throughput screening (HTS) formats. At low sample volumes, the QTL microsphere suspensions will be replaced with glass slides or nanochips or microplate wells with QTL polymer deposited on the surface.

How much time does it take for a QTL experiment? How fast is the QTL assay?

QTL assay is a true “mix-and-measure” assay for most applications. The incubation time required for the mixture is less than a minute. The QTL Assay is very rapid since the processes involved are high fidelity and therefore occur instantaneously. In a typical competition QTL assay, “hands on” tech-time is about 5 minutes. With materials ready, a calibration curve could be generated within 30-60 minutes depending on the amount of enzyme utilized. Single point measurements take 1-20 minutes, but samples could be “batched” and analyzed simultaneously, thereby reducing overall assay time. The steps involved in a typical QTL assay are: mixing the QTL and the target sample, exposing the QTL polymer to the mixture, then measuring light emission from the mixture immediately.

What type and how much enzyme are utilized in the assay?

The assay provides human recombinant BACE-1 (β -secretase) enzyme of approximate molecular weight of 73 kDa. The assay provides excellent signal over background in 60 minutes using just 5 nanograms of enzyme.

Can I use my own source of enzyme?

The assay is compatible with multiple sources of enzyme, however, for best results, we recommend the use of enzyme supplied by QTL Biosystems.

What is the signal to background?

The signal to background is the ratio of the total signal measured in the assay in the presence of enzyme activity to the signal measured in the absence of any enzyme activity.

What is the signal to background for the assay?

The signal to background was measured at 1.70 at approximately 20% conversion of the substrate.

What is signal to noise? How do you measure it?

The signal to noise (S/N) ratio can be considered to be the “signal strength” of the assay divided by the average variability of the screen. The formula to use to calculate the S/N ratio follows:

$$S/N = \frac{(\text{Mean Pos Control} - \text{Mean Neg Control})}{\sqrt{(\text{Pos Control STD})^2 + (\text{Neg Control STD})^2}}$$

where Mean Pos Control is the signal in the presence of enzyme activity and the Mean Neg Control is the background signal in the absence of enzyme activity. The denominator contains the standard deviations of the values.

What is the signal to noise of the assay?

The signal to noise was measured at 16 at approximately 20% conversion of the substrate.

What is the Z-factor associated with the assay?

The Z'-factor, which is a measure of the assay consistency, has been measured to be above 0.70 at 20% conversion of the peptide substrate. Use of automated reagent dispensing is expected to improve the consistency and hence the Z'-value.

What is the co-efficient of variation for the assay?

The CV for the assay has been determined to be less than 2%

What is the Km?

The Michaelis-Menten Constant, Km is a measure of the binding affinity of a substrate to an enzyme. For more information, please refer to introductory biology texts.

What is the DMSO tolerance level of the QTL assay?

DMSO tolerance of the QTL assay is likely to be dependent upon the particular target/capture system under investigation. For small molecule targets devoid of any long-range secondary, tertiary, or quaternary structure (example, biomolecules), the assay components should tolerate high levels of DMSO. In the drug development industry sector, typical levels of DMSO tested range from 1-25% (v/v). There are no inherent negative performance consequences to the QTL polymeric sensors that would preclude their use in DMSO. The QTL Assay tolerates DMSO to a much higher level than most other assays on the market. This can be attributed to the robustness of the QTL polymer and the QTL sensor. At QTL Biosystems, QTL reagents are synthesized using organic solvents; thus they are quite stable in that medium.

What is the thermal/pH stability of QTL polymers? What is the thermal/pH stability of the QTL polymer coatings?

The pH of the QTL polymer coating can be varied to suit different applications. For example, if an adsorptive coating of negatively charged QTL conducting polymer is desired, then a pH of 4 or lower could be used. Alternately, the same negatively charged QTL polymer could be specifically adsorbed through, for example, biotin-avidin interactions (biotinylated QTL polymer coated onto streptavidin or neutravidin-coated microspheres). In this case, a neutral pH is required during QTL polymer deposition. The stability of the QTL polymer coating on microspheres is quite resistant to acute heating (70°C for 10 minutes) as well as exposure to high protein concentrations (3% BSA, w/v).

What are the emission wavelengths of the QTL polymers?

The emission wavelengths of the QTL polymers depend upon the molecular structure of the polymer. QTL polymers are normally conducting polymers that make use of all of the visible range of light emission (400 – 800 nm). QTL Biosystems is actively engaged in expanding this emissive range to include UV and IR regions of the spectrum. A significant portion of the QTL polymer coatings employs electrostatic adsorption that result in non-aggregated emission around 480 nm. Other QTL polymer coatings could have an aggregate character to the emission, centered at 530 nm.

What is the linear range of the QTL assay?

This would vary depending upon the target analyte and the type of QTL assay (competitive or displacement or enzyme catalysis). The linear range is best illustrated with actual examples of QTL assays. The linear range of the DNA detection QTL assay is 0.5 pmol to 50 pmol. The linear range of the QTL Ovalbumin assay is between 5-50pmol. However, a linear range of 2 to 3 orders of magnitude in target concentration is achievable with most QTL assays.

Is the QTL assay homogeneous?

Yes. QTL assays are true “mix-and-measure” assays. On occasion, QTL Biosystems could also develop heterogeneous QTL assays in order to meet specific customer requirements, to improve sensitivity and/or test specificity or enhance the detection dynamic range.

Which compounds show interference in the assay?

In our limited screen of various organic small molecules, biological proteins and inorganic salts, we have not come across any significant interferents at concentrations that are important for high throughput screening.

What types of inhibitors may be incompatible with the assay?

Potentially, compounds that quench the fluorescence of the sensor, or react with the peptide substrate are incompatible with the assay. We do not have any specific examples of such materials.

What is the stability of the reagents?

If all reagents are stored under recommended conditions, the reagents should be stable over a period of three months.

What is the shelf life of the reagents?

The shelf life of all reagents under proper recommended storage conditions is three months.

Can the assay reagents be dispensed manually or robotically?

The assay reagents including the microsphere sensor can be dispensed either manually using a pipette or using automated dispensers.

What are the storage conditions for the reagents?

The peptide and enzyme as supplied should be stored at -80°C, the peptide also protected from light. The sensor and assay buffer as supplied should be stored at 4°C, the sensor protected from light.

Do any of the kit components require special handling or storage?

The peptide and sensor need to be protected from light as much as possible. These reagents will degrade over long-term storage under visible light; however, can be handled under normal light for very short periods. Reagents should not be warmed above ambient room temperature.