# An Ultra-Sensitive Fluorimetric Assay for the Detection of Renin Activity

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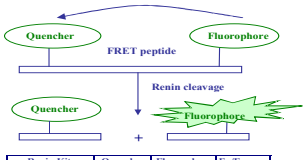
## Introduction

Renin plays a key role in the regulation of renin-angiotensin system. Renin acts by cleaving the substrate, angiotensinogen, to angiotensin I in the blood, where it is further converted into angiotensin II. Since an overactive renin-angiotensin system leads to hypertension, renin is an attractive target for the treatment of this disease. In the past decade, a considerable number of structurally different synthetic renin inhibitors of excellent potency have been described. To facilitate high throughput screening of renin inhibitors, we have developed new renin assay kits utilizing FRET peptide substrates. The SensoLyte™ 520 Renin Assay Kit contains FRET peptide labeled with the quencher QXL™520 and the fluorophore 5-FAM. For the Sensolyte™ 390 Renin Assay Kit we used a Dnp/Amp FRET pair with the sequence Dnp-Lys-His-Pro-Phe-His-Leu-Val-Ile-His-L-Amp, based on published data.[1] The sequence of the QXL™ 520/5-FAM FRET substrate was modified. These newly developed assays were validated with several known renin inhibitors. Both assays showed good sensitivity, although the QXL™520/5-FAM substrate was significantly superior to both the Dnp/Amp substrate and a previously reported EDANS/DABCYL substrate with sequence Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(DABCYL)-Arg.

## Results




| Renin Kits | Quencher | Fluorophore | Ex/Em, nm |
|---|---|---|---|
| Sensolyte™ 520 | QXL™ 520 | 5-FAM | 490/520 |
| Sensolyte™ 390 | Dnp | Amp | 330/390 |
| Competitor assay | DABCYL | EDANS | 340/490 |

**Figure 1. The principle of FRET based enzyme assays.** In the intact FRET peptide, fluorescence is quenched. After cleavage into two separate fragments by renin, the fluorescence is recovered and can be monitored.

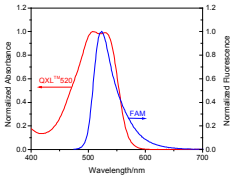



**Figure 2. 5-FAM and QXL™520 is a new donor - acceptor pair for FRET peptides.** QXL™520 is an excellent quencher when paired with 5-FAM. Upon cleavage into two separate fragments by renin, the fluorescence of 5-FAM can be monitored at Ex/Em = 490/520 nm.

### Advantages of QXL™ 520 - 5-FAM FRET pair:

- The absorption spectrum of QXL™ 520 overlaps with the emission spectrum of 5-FAM.
- Hydrophility of QXL™ 520 results in better solubility of the peptide substrate.
- Higher brightness and stability of 5-FAM compared with EDANS and Amp.
- Long wavelength fluorescence of 5-FAM is less interfered by autofluorescence of drug candidates.

For these newly designed FRET peptides, initial rates of hydrolysis by enzyme were determined after incubation of renin (135 ng/ml) with a range of concentrations of substrates at 37°C. The resulting data were analyzed by linear regression.

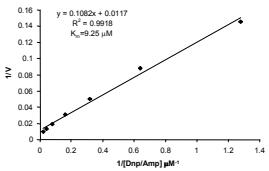



**Figure 3A. Lineweaver-Burk plots for renin with Dnp/Amp FRET peptide as substrate.**

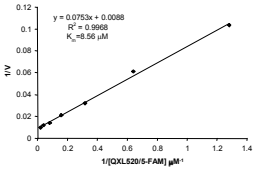



**Figure 3B. Lineweaver-Burk plots for renin with QXL™520/5-FAM FRET peptide as substrate.**

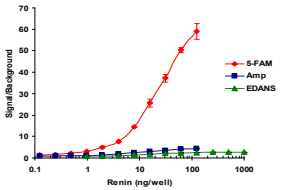



**Figure 4. Assay comparison with different enzyme concentrations.** Signal increases up to 60 fold after 30 minutes of renin incubation with QXL™520/5-FAM FRET substrate. In comparison to only 2.5 fold signal increase observed for an existing EDANS/DABCYL based assay. A 4-fold increase is observed for the Dnp/Amp renin FRET substrate.

To further validate renin activity assay, $IC_{50}$ was determined for the renin inhibitor, derived from sequence Z-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(Boc)-OMe described in the literature.[2]

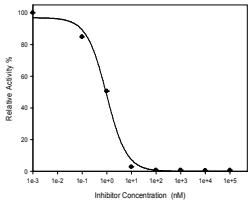



**Figure 5. Inhibition of renin activity with SensoLyte™ 520 Assay Kit.** QXL™520/5-FAM substrate (7 mM) was incubated with 3.5 nM renin in the presence of inhibitor ($10^{-3}$ to $10^{5}$ nM). Kinetic readings were taken every 3 minutes for 15 minutes at 37°C (Flexstation 384II, Molecular Devices). Calculated $IC_{50}$ was 0.98 nM. The SensoLyte™ 390 Assay Kit had approximately the same $IC_{50}$(Data are not shown).

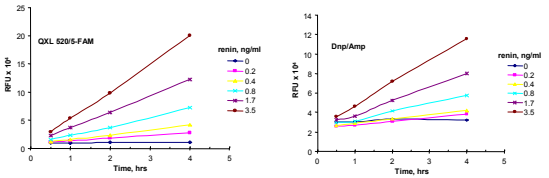



**Figure 6. Renin titration with SensoLyte™ 520 and SensoLyte™ 390 Assay Kits.** FRET peptide substrates were incubated with the indicated amount of enzyme at 37°C and fluorescence signals were measured over time (Flexstation 384II, Molecular Devices). Sensitivity of the SensoLyte™ 520 Renin Assay Kit was 0.8 ng/ml renin with 30 minutes incubation time and 0.2 ng/ml with 2 hours incubation time. SensoLyte™ 390 assay detected 0.8 ng/ml renin with 2 hours incubation time.

### Summary of the renin assay kits

| | 520 Renin Assay | 390 Renin Assay | Competitor Assay |
|---|---|---|---|
| **FRET pair** | QXL™ 520/5-FAM | Dnp/Amp | EDANS/DABCYL |
| **Autofluorescence** | Minimal | Moderate | Moderate |
| **Signal/Background*** | 25 | 3 | 2 |
| **Enzyme sensitivity**** | 0.8 ng/ml | 8 ng/ml | 32 ng/ml |
| **Z'** | 0.74 | 0.61 | N/A |

*Note: Specific activity of enzyme was determined to be 135 pmoles of EDANS/DABCYL substrate hydrolyzed in min/mg and 500 pmoles of QXL™520/5-FAM substrate hydrolyzed in min/mg.*

*with 0.13 ng/ml enzyme, with 30 min. incubation

**with 30 min. incubation

## Conclusions

- We have developed sensitive renin activity assay kits including an ultra-sensitive renin assay based on a QXL™ 520/5-FAM FRET substrate.
- Assay with QXL™ 520/5-FAM substrate was approximately 40 fold more sensitive than an existing assay which uses an EDANS/DABCYL FRET substrate and 10 fold more sensitive than the Dnp/Amp based assay.
- The longer excitation and emission wavelengths 5-FAM minimize the interference from the autofluorescence of test compounds.
- The newly developed assays can be used to detect renin activity and can be applied to the high throughput screening of renin inhibitors.

## References

1. Paschalidou, K. et al. *Biochem. J.* **382**, 1031 (2004).
2. Wood, JM. et al. *Hypertension* **7**, 797 (1985).