



SensoLyte[®] 520 Renin Assay Kit **Fluorimetric**

Catalog #	AS-72040
Kit Size	100 Assays (96-well plate)

- **Convenient Format:** Complete kit including all the assay components.
- **Optimized Performance:** Optimal conditions for screening of renin inhibitors.
- **Enhanced Value:** Less expensive than the sum of individual components.
- **High Speed:** Minimal hands-on time.
- **Assured Reliability:** Detailed protocol and references are provided

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Renin substrate 5-FAM/QXL [™] 520 FRET peptide Ex/Em=490 nm/520 nm upon cleavage	1.5 mM DMSO solution, 50 μL
Component B	5-FAM, fluorescence reference standard	1mM, 20 μL
Component C	Human recombinant renin	50 μL
Component D	Assay buffer	25 mL
Component E	Renin Inhibitor Ac-HPFV- (Sta)-LF-NH ₂	1mM DMSO solution, 4 μL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom 96-well with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.

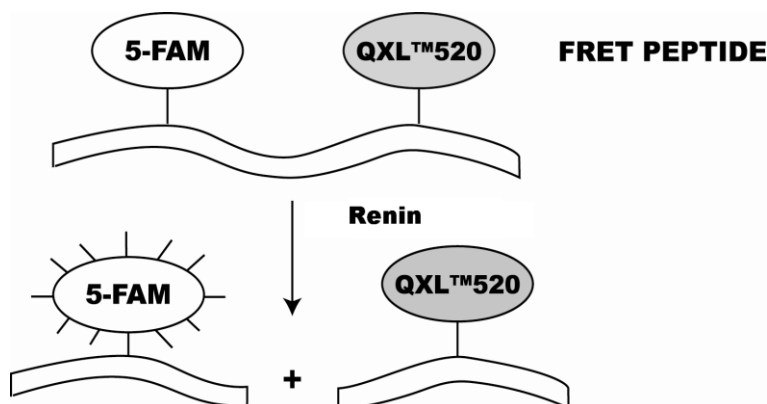
Storage and Handling

- Store all kit components at -20°C except for Component C, which should be stored at -80°C
- Protect Components A and B from light
- If used frequently, Component D can be stored at room temperature for convenience.

Introduction

The renin–angiotensin system (RAS) plays a central role in the regulation of blood pressure and electrolyte homeostasis.¹ At the first and rate-limiting step of the RAS cascade, renin (EC 3.4.23.15), a highly specific aspartyl protease, cleaves angiotensinogen, produced in the liver, to yield angiotensin I, which is further converted into angiotensin II by ACE (Angiotensin Converting Enzyme). Angiotensin II constricts blood vessels leading to increased blood pressure. It also increases the secretion of ADH and aldosterone, and stimulates the hypothalamus to activate the thirst reflex. 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 (sub-nanomolar) potency and selectivity have been described,²⁻⁴ however, to accelerate the drug-discovery process and to perform automated inhibitor screening, a continuous, homogeneous assay with picomolar sensitivity is needed.

The SensoLyte[®] 520 Renin Assay Kit provides a convenient assay for high throughput screening of renin inhibitors and for continuous assay of renin activity using a 5-FAM/QXL[™]520 fluorescence resonance energy transfer (FRET) peptide. The sequence of this FRET peptide is derived from the cleavage site of renin.⁵ In the FRET peptide the fluorescence of 5-FAM is quenched by QXL[™]520. Upon cleavage into two separate fragments by renin (**Scheme 1**), the fluorescence of 5-FAM is recovered, and can be monitored at excitation/emission = 490/520 nm. With a high fluorescence quantum yield and long emission wavelength, the signal of 5-FAM can be detected with less interference from the autofluorescence of cell components and test compounds. The assay can detect as low as 0.8 ng/ml active renin. The assays are performed in a convenient 96-well microplate format.



Scheme 1. Proteolytic cleavage of 5-FAM/QXL[™]520 FRET peptide by renin.

Protocol

Screening renin inhibitors using recombinant enzyme

Note 1: Warm all kit components until thawed to room temperature before starting the experiments.

Note 2: For standard curve, please refer to Appendix II (optional).

1. Prepare working solutions.

1.1 Renin substrate solution: Dilute renin substrate (Component A) 1:100 in assay buffer (Component D) according to Table 1. For each experiment prepare fresh substrate solution.

Table 1. Renin substrate solution for one 96-well plate (100 assays)

Components	Volume
Renin substrate (100X, Component A)	50 μ L
Assay buffer (Component D)	4.95 mL
Total volume	5 mL

1.2 Renin diluent: Dilute renin (Component C) 1:80 in assay buffer (Component D). This amount of enzyme is enough for a full 96-well plate. If not using the entire plate, adjust the amount of enzyme to be diluted accordingly.

Note: Prepare enzyme diluents immediately before use. Do not vortex the enzyme solutions. Prolonged storage or vigorous agitation of the diluted enzyme will cause denaturation. Store the enzyme solution on ice.

1.3 Renin inhibitor:⁶ Dilute the 1 mM inhibitor solution (Component E) to 10 μ M in assay buffer (Component D). Add 10 μ L (96-well plate) of the 10 μ M inhibitor solution into each of the inhibitor control well (DMSO concentration should not exceed 1%).

2. Set up enzymatic reaction.

2.1 Add test compounds and renin solution into the microplate wells. The suggested volume of renin solution for one well of a 96-well plate is 40 μ L and test compound is 10 μ L.

2.2 Set up the following controls at the same time as deemed necessary:

- Positive control contains diluted renin without test compound.
- Inhibitor control contains diluted renin and diluted renin inhibitor (provided as undiluted in the kit).
- Vehicle control contains diluted renin and diluted vehicle used to deliver test compound (e.g. DMSO, concentration not to exceed 1%).
- Test compound control contains assay buffer and test compound. Some test compounds have strong autofluorescence and may give false results.
- Substrate control contains assay buffer.

Note: Using the assay buffer, bring the total volume of all controls to 50 μ L (96-well plate).

3. Pre-incubation.

3.1 Incubate the plate at 37°C for 30 min. At the same time, also incubate the renin substrate solution at 37°C.

4. Initiate the enzymatic reaction.

4.1 Add 50 μL (96-well plate) of renin substrate solution into each well. Mix the reagents completely by shaking the plate gently for no more than 30 seconds.

4.2 Measure fluorescence signal:

- **For kinetic reading:** Immediately start measuring fluorescence intensity at $\text{Ex/Em}=490\text{ nm}/520\text{ nm}$ continuously and record data every 1 min. for 15 min (37°C recommended).
- **For end-point reading:** Incubate the reaction at 37°C for 15 min. Keep plate from direct light. Then measure fluorescence intensity at $\text{Ex/Em}=490\text{ nm}/520\text{ nm}$.

4.3 Data analysis: Refer to Appendix I.

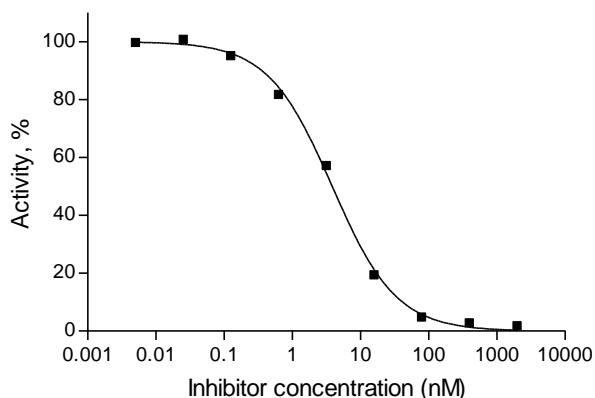


Figure 1. Inhibition of renin activity by renin inhibitor, Ac-HPFV-(Sta)-LF-NH₂, as measured with SensoLyte[®] 520 Renin Assay Kit.

Appendix I: Data Analysis

- The fluorescence reading from the substrate control well is the background fluorescence. This background reading should be subtracted from the readings of the other wells. The fluorescence readings are expressed in relative fluorescence units (RFU).
- For kinetic reading:
 - Plot data as RFU versus time for each sample. If you want to convert the RFU to the concentration of the product of enzymatic reaction, please refer to [Appendix II](#) for setting up a fluorescence reference standard.
 - Determine the range of initial time points during which the reaction is linear. Typically, 10-15% conversion will be the optimal range.
 - Obtain the initial reaction velocity (V_o) in RFU/min. Determine the slope of the linear portion of the data plot.
 - A variety of data analyses can be done, e.g., determining inhibition %, EC_{50} , IC_{50} , K_m , K_i , etc.
- For endpoint reading:
 - Plot data as RFU versus concentration of test compounds.

- A variety of data analyses can be done, e.g., determining inhibition %, EC_{50} , IC_{50} , etc.

Appendix II: Instrument Calibration

- **5-FAM fluorescence reference standard:** Dilute 1000 μM 5-FAM (Component B) to 14 μM in assay buffer (Component D). Do 2-fold serial dilutions to get concentrations of 7, 3.5, 1.75, 0.875, 0.437, and 0.218, include an assay buffer blank. Add 50 μL /well (96-well plate) of these serially diluted 5-FAM reference solutions.
- Add 50 μL /well (96-well plate) of the diluted renin substrate solution (refer to Step 1.1 for preparation).
Note: The renin substrate solution is added to the 5-FAM reference standard to correct for the absorptive quenching by the FRET peptide. If multiple concentrations of substrate are used, this step must be performed for each concentration.
- Measure the fluorescence intensity of the reference standard wells at $Ex/Em=490/520$ nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot the 5-FAM fluorescent reference standard curve as RFU (relative fluorescent units) versus concentration as shown in Figure 2.

Note: The final concentrations of 5-FAM reference standard are 7, 3.5, 1.75, 0.875, 0.437, 0.218, 0.109, and 0 μM . This reference standard is used to calibrate the variation of different instruments and different experiments. It is also an indicator of the amount of final product of the renin enzymatic reaction.

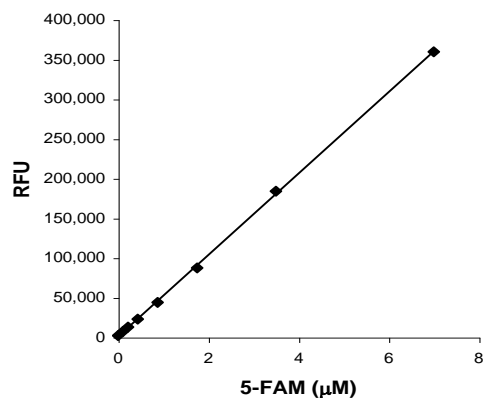


Figure 2. 5-FAM reference standard. 5-FAM was serially diluted in assay buffer containing substrate, and the fluorescence recorded at $Ex/Em=485\pm 20$ nm/ 528 ± 20 nm. (Flexstation 384II, Molecular Devices)

References

1. He, FJ. et al., *J. Renin Angiotensin Aldosterone Syst.* **4**, 11 (2003).
2. Wood, JM. et al., *Hypertension*, **7**, 797 (1985).
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4. Wood, JM. et al., *Biochem. Biophys. Res. Comm.* **308**, 698 (2003).
5. Paschalidou, K. et al., *Biochem. J.* **382**, 1031 (2004).
6. Hui, KY. et al., *J. Med. Chem.* **31**, 1679 (1988).