# SensoLyte® Rh110 Furin Activity Assay Kit *Fluorimetric*

| Catalog # | AS-72256 |
|---|---|
| **Kit Size** | 100 Assays (96-well plate) |

- ***Optimized Performance:*** This kit is optimized to detect furin enzyme activity.
- ***Enhanced Value:*** It provides ample reagents to perform 100 assays in a 96-well plate format.
- ***High Speed:*** The entire process can be completed in one hour.
- ***Assured Reliability:*** Detailed protocol and references are provided.

## Kit Components, Storage and Handling

| Component | Description | Quantity |
|---|---|---|
| Component A | Rh110 furin substrate, Ex/Em=490/520 nm upon cleavage | 0.6 mM, 50 µL |
| Component B | Rh110, fluorescence reference standard, Ex/Em=490/520 nm | 0.6 mM, 10 µL |
| Component C | Recombinant human furin | 10 µg/mL, 10 µL |
| Component D | 2X Assay Buffer | 20 mL |
| Component E | Inhibitor | 0.1 mM, 10 µL |

### Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom, 96-well plate 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.
- Store Component C at -80°C.
- Protect Components A and B from light and moisture.
- Component D can be stored at room temperature for convenience.

## Introduction

Furin is a serine protease that belongs to the subtilisin-like proprotein convertase (PC) family. Furin catalyzes the proteolytic maturation of proprotein substrates in the secretory pathway. It plays an essential role in embryogenesis and homeostasis, as well as in diseases ranging from Alzheimer's disease, cancer, anthrax, to Ebola fever. Furin is essential to the maturation and viral infectivity of HIV. Furin is a potential target for drug design, especially for the inhibition of furin-dependent virus replication.

The SensoLyte® Rh110 Furin Assay Kit provides a convenient assay for screening of enzyme inhibitors or for continuous assay of furin activity using a fluorogenic substrate. Upon cleavage by furin, this substrate generates the Rh110 (rhodamine 110) fluorophore with bright green fluorescence that can be detected at excitation/emission=490 nm/520 nm. The longer-wavelength spectra and higher extinction coefficient of the Rh110 provide greater sensitivity and allows for less interference from the other reaction components. The detection limit can reach as low as 0.02 ng/mL.

## Protocol

Note 1: To prepare a standard curve, please refer to Appendix II (optional).

**Screening compounds using purified enzyme.**

**1. Prepare working solutions.**

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

1.1 1X assay buffer: Add 10 mL of 2X assay buffer (Component D) to 10 mL of deionized water.

1.2 Furin substrate solution: Dilute furin substrate (Component A) 100-fold in 1X assay buffer. Refer to Table 1. For each experiment, prepare fresh substrate solution. If not using the entire plate, adjust the amount of substrate to be diluted accordingly.

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

| Components | Volume |
|---|---|
| Furin substrate (Component A) | 50 µL |
| 1X assay buffer | 4.95 mL |
| Total volume | 5 mL |

1.3 Furin enzyme diluents: Dilute furin (Component C) 400-fold in 1X assay buffer. 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 solution. Prolonged storage or vigorous agitation of the diluted enzyme will cause denaturation. Store the enzyme solution on ice.

1.4 Furin inhibitor, a peptidyl chloromethylketone (Decanoyl-Arg-Val-Lys-Arg-CMK): Dilute the 0.1 mM inhibitor solution (Component E) 100-fold in 1X assay buffer to obtain 1µM diluted inhibitor solution. Add 10 µl of the diluted inhibitor solution into each of the inhibitor control well.

**2. Set up the enzymatic reaction.**

2.1 Add test compounds and diluted enzyme solution to the microplate wells. The suggested volume of enzyme solution for one well of a 96-well plate is 40 µL and test compound is 10 µL.

2.2 Simultaneously set up the following control wells, as deemed necessary:

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

2.3 Using the 1X assay buffer, bring the total volume of all controls to 50 µL.

2.4 Optional: Pre-incubate the plate for 10 min. at assay temperature. Any temperature (the *assay temperature*) from room temperature to 37°C may be used, as long as the subsequent incubations are performed at the same temperature.

**3. Run the enzymatic reaction.**

3.1 Add 50 µL of the furin substrate solutions into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence at Ex/Em=490nm/520 nm continuously and record data every 5 min for 30 to 60 min.
- For end-point reading: Incubate the reaction for 30 to 60 min. Keep plate away from direct light. Mix the reagents and measure fluorescence intensity at Ex/Em=490nm/ 520nm.

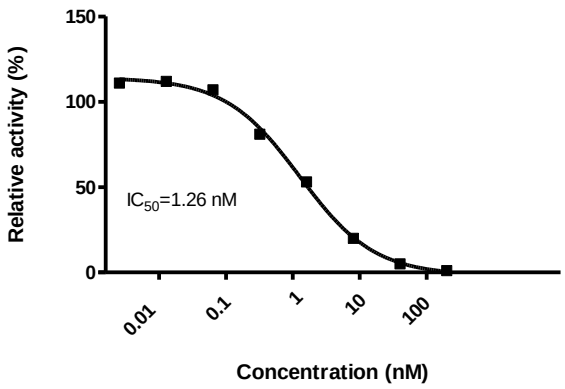


Figure 1.
Inhibition of furin activity by a peptidyl chloromethylketone measured with SensoLyte® Rh110 Furin Assay Kit. (SpectraMax M5 Microplate Reader, Molecular Devices)

3.3 For methods of data analysis: Refer to Appendix I.

## Appendix I. Data Analysis

The fluorescence reading from the non-cell control well or substrate control well is the background fluorescence. This background reading has to be subtracted from the readings of the other wells to obtain the relative fluorescence unit (RFU).

- For kinetics reading:

➢ Plot data as RFU versus time for each sample.
➢ Determine the range of initial time points during which the reaction is linear. 10-15% conversion is the optimal range.
➢ Obtain the initial reaction velocity (Vo) 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 the 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

- Fluorescence reference standard: Dilute the 0.6 mM fluorescence standard solution (Component B) 100-fold to 6 μM in 1X assay buffer. Do 2-fold serial dilutions to obtain the following concentrations: 3, 1.5, 0.75, 0.375, 0.188, and 0.094, include an assay buffer blank. Add 50 μL/well of these serially diluted reference solutions.
- Add 50 μL/well of the diluted furin substrate solution (refer to Step 1.2 for preparation).

  Note: The furin substrate solution is added to the reference standard to normalize for the intrinsic substrate fluorescence. If multiple concentrations of substrate are used, this step must be performed for each concentration.
- Measure the fluorescence of the reference standard and substrate control wells at Ex/Em=490 nm/520 nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot the reference standard curve as RFU (relative fluorescent units) versus concentration.
- The final concentrations of fluorescence reference standard are 3, 1.5, 0.75, 0.375, 0.188, 0.094, 0.047 and 0 μM. This reference standard is used to calibrate the variation in different instruments and different experiments. Since the proteolytic cleavage of the Rh110 substrate consists of two steps, with both the intermediate and final products having fluorescence, the Rh110 reference standard cannot serve as an indicator of the amount of enzymatic reaction final product.

## References


1. Van den Ouweland, A.M. et al. *Nucleic Acids Res.* **18**, 664 (1990).
2. Watanabe T. et al. *J. Biol. Chem.* **267**:8270 (1992).
3. Molloy S.S. et al. *J. Biol. Chem.* **267**: 16396 (1992).
4. Thomas, G. et al. *Nature Rev. Mol. Cell Biol.* **3**:753 (2002) .
5. Dahms S. O. et al. *PNAS* **113**:11196 (2016).


*Revision 1.0*