



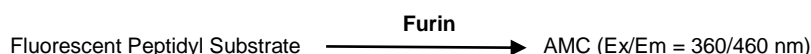
Furin Inhibitor Screening Kit (Fluorometric)

rev 01/21

(Catalog # K2069-100; 100 assays; Store at -20 °C)

I. Introduction:

Furin is a serine protease that belongs to the subtilisin-like Proprotein Convertases (PCs) family. PCs family members are calcium-dependent serine endoproteases that process latent, immature precursor proteins into their biologically active, functional form. Furin is predominantly localized in the Golgi apparatus, where it functions to cleave other proteins into their mature, active forms. However, it cycles between Golgi, cell surface, and the endosomes. Furin plays an important role in numerous processes including cell survival, migration, maintenance of homeostasis, embryogenesis, and in diseases. In addition to processing precursor proteins, Furin is utilized by numerous viral and bacterial pathogens for enhancing their virulence and spread. For example, the envelope protein of viruses such as HIV, influenza, several filoviruses including Ebola and Marburg virus, and the spike protein of SARS-CoV-2 must be cleaved by furin or furin-like proteases to become fully functional. Because of the role of Furin in many cellular processes and diseases, it is emerging as a potential therapeutic target for infectious and non-infectious diseases. **BioVision's Furin Inhibitor Screening Kit** provides a quick, easy and sensitive assay for screening, studying and characterizing potential inhibitors of Furin. In this assay, Furin cleaves a fluorescent peptidyl substrate and generates a fluorescence signal, which is detected at Ex/Em = 360/460 nm. A potent, specific Furin inhibitor is also included in the kit.



II. Application:

- Screening or characterizing Furin inhibitors.

III. Kit Contents:

Components	K2069-100	Cap Code	Part Number
Furin Assay Buffer	25 ml	WM	K2069-100-1
Furin Substrate	25 μ l	Red	K2069-100-2
Furin, Human Recombinant	40 μ l	Blue	K2069-100-3
Furin Inhibitor (1 mM)	25 μ l	Orange	K2069-100-4

IV. User Supplied Reagents and Equipment:

- DMSO
- 96-well white plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- Furin Assay Buffer & Furin Substrate:** Warm to room temperature (RT) before use.
- Furin, Human Recombinant:** Thaw on ice. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Use diluted Recombinant Furin for the assay.
- Furin Inhibitor (1 mM in DMSO):** Warm to RT. Divide into aliquots and store at -20 °C. Prepare 1:10 dilution of the 1 mM Furin Inhibitor in DMSO (not provided) to make 100 μ M Furin Inhibitor. Diluted Furin Inhibitor can be aliquoted and stored at -20 °C. Avoid repeated freeze/thaw cycles.

VI. Furin Inhibitor Screening Protocol:

1. Furin, Human Recombinant: Prepare 1:20 dilution of Furin, Human Recombinant using Furin Assay Buffer. Mix thoroughly and keep on ice. Add 8 μ l of diluted Furin into the desired wells of a 96-well white plate labeled as **Sample**, **Solvent Control**, **Inhibitor Control** and **Enzyme Control**. Adjust the volume of all wells to 25 μ l using Furin Assay Buffer.

2. Screening Test Inhibitor(s): Dissolve Test Inhibitor(s) in an appropriate solvent to make 100X stock solution. Dilute the stock Test Inhibitor to 4X using Furin Assay Buffer. Add 25 μ l of diluted Test Inhibitor into the Sample well(s). Add 25 μ l of 4X Solvent (4X final well solvent concentration) into the **Solvent Control** well. **Note:** Solvents used to solubilize the Test Inhibitor(s) might affect the enzymatic activity. Thus, prepare a Solvent Control well with the same final concentration of solvent used to dissolve the Test Inhibitor(s).

3. Enzyme Control, Background Control and Inhibitor Control Preparation: Add 25 μ l of Furin Assay Buffer to the **Enzyme Control** well. For **Background Control**, add 50 μ l of Furin Assay Buffer in a separate well. To the **Inhibitor Control** well, add 2 μ l of 100 μ M Furin Inhibitor and adjust the volume to 50 μ l/well by adding 23 μ l Furin Assay Buffer. At this stage, all wells including Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control contains 50 μ l/well. Incubate for 30 min at RT, protected from light.

IC₅₀ estimation (Optional): Prepare several dilutions of the Test Inhibitor(s) in Furin Assay Buffer while maintaining the consistent final Solvent Concentration in all wells. Add 25 μ l of each dilution into the designated wells.

4. Furin Substrate Mix Preparation: Mix enough Substrate Mix for the number of assays to be performed. Prepare 50 μ l Substrate Mix per reaction as shown below.

	<u>Substrate Mix</u>
Furin Assay Buffer	49.8 μ l
Furin Substrate	0.2 μ l

