



# **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 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 µl	Red	K2069-100-2
Furin, Human Recombinant	40 µl	Blue	K2069-100-3
Furin Inhibitor (1 mM)	25 µl	Orange	K2069-100-4

# IV. User Supplied Reagents and Equipment:

- DMSC
- 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 μM 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 µl 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 µl 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 µl of diluted Test Inhibitor into the Sample well(s). Add 25 µl 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 µl of Furin Assay Buffer to the Enzyme Control well. For Background Control, add 50 µl of Furin Assay Buffer in a separate well. To the Inhibitor Control well, add 2 µl of 100 µM Furin Inhibitor and adjust the volume to 50 µl/well by adding 23 µl Furin Assay Buffer. At this stage, all wells including Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control contains 50 µl/well. Incubate for 30 min at RT, protected from light.

*IC*<sub>50</sub> 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 μl 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 μl Substrate Mix per reaction as shown below.

Cubatrata Mix

	Substrate with
Furin Assay Buffer	49.8 µl
Furin Substrate	0.2 µl



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Add 50 µl Substrate Mix to Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control wells. The total reaction volume is 100 µl/well.

5. Measurement: Measure fluorescence in kinetic mode at Ex/Em = 360/460 nm at 5 min interval for 30-60 min at RT.

6. Calculation: Obtain  $\Delta$ RFU for all Test Samples [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control [IC] by subtracting RFU at time  $t_1$  from RFU at time  $t_2$ , such that  $t_2$  and  $t_1$  is within a linear range of the assay. Calculate the slope for all Samples including Enzyme Control [EC] by dividing  $\Delta$ RFU by time  $\Delta$ t ( $t_2$  -  $t_1$ ). If [SC] slope is significantly different from [EC] slope, use its values instead of EC in the calculations shown below.

% Relative Inhibition = 
$$\frac{\text{Slope of [EC]} - \text{Slope of [S]}}{\text{Slope of [EC]}} X 100$$

Note: Subtract the reading of [BC] wells from all [S], [EC], and [SC] wells.

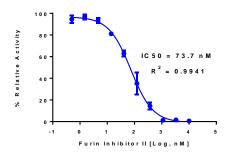


Figure: Inhibition of Furin activity by Furin inhibitor. IC50 was calculated to be 73.7 nM. Assay was performed following the kit protocol.

## VII. Related Products:

Furin, Human Recombinant (Cat. # P1658-10, 50)

Furin/PCSK3, Human CellExp™, human recombinant (Cat. # 7249-10)

Furin/PACE Polyclonal Antibody (Cat. # 3873-30T, 100)

Furin/PACE Blocking Peptide (Cat. # 3873BP-50)

Human CellExp™ BACE1, human recombinant (Native) (Cat. # 7398-10, 50)

Active Recombinant Human beta-Secretase 1 (BACE-1) (Cat. # 7609-5, 50, 1000)

Human CellExp™ Recombinant Ebolavirus BDBV Small/secreted Glycoprotein (sGP) (Cat. # P1059-10, 50)

Human CellExp™ Recombinant Ebolavirus BDBV Envelope Glycoprotein 1 (GP1) (Cat. # P1061-10, 50)

Human CellExp™ Recombinant EBOV Envelope Glycoprotein 1 (Cat. # P1060-10, 50)

β-Secretase (BACE1) Activity Assay Kit II (Fluorometric) (Cat. # K388-100)

Human β-Secretase (BACE1) Inhibitor Screening Kit (Fluorometric) (Cat. # K720-100)

FOR RESEARCH USE ONLY! Not to be used on humans.

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