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Furin Activity Assay Kit (Fluorometric)

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(Catalog # K2076-100; 100 assays; Store at -20 °C)

I. Introduction:

Furin is a serine protease that belongs to the Proprotein Convertase Subtilisin (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. Relatively little is known about the activity of Furin in humans. Understanding the activity levels of Furin in human biology could lead to a better understanding of its interaction with other PCs, trafficking itinerary and its role in human diseases. **BioVision's Furin Activity Assay Kit** provides a quick, easy and sensitive method to determine Furin activity in various samples. In this assay, Furin activity is measured in the presence or absence of Furin Inhibitors. The difference between the fluorescence obtained in the presence or absence of Furin Inhibitors represents the actual Furin activity. A potent, specific Furin inhibitor is also included in the kit.

$$\text{Fluorescent Peptidyl Substrate} \xrightarrow{\textbf{Furin}} \text{AMC (Ex/Em = 360/460 nm)}$$

II. Application:

- To determine Furin activity in various samples.

III. Sample Types:

- Plasma, Serum etc.

IV. Kit Contents:

Components	K2076-100	Cap Code	Part Number
Furin Assay Buffer	25 ml	WM	K2076-100-1
Furin Substrate	25 µl	Red	K2076-100-2
Furin Positive Control	10 µl	Blue	K2076-100-3
Furin Inhibitor (1 mM)	25 µl	Orange	K2076-100-4
Deactivator (50 mM)	200 µl	Amber	K2076-100-5
AMC Standard	100 µl	Yellow	K2076-100-6

V. User Supplied Reagents and Equipment:

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

VI. 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 & AMC Standard (1 mM):** Ready to use. Warm to room temperature (RT) before use.
- **Furin Positive Control:** Thaw on ice. Prepare 1:10 dilution of Furin Positive Control using Furin Assay Buffer. Divide the diluted Furin Positive Control into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles. Use diluted Furin Positive Control 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.
- **Deactivator (50 mM in DMSO):** Warm to RT. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

VII. Furin Activity Assay Protocol:

1. Serum & Plasma Preparation: Store Serum & Plasma at -80 °C to avoid loss of bioactivity and contamination. Sample to be used within 5 days may be stored at -20 °C. Avoid multiple freeze-thaw cycles. Centrifuge sample(s) at 12000 x g and 4 °C for 10 min. Collect the supernatant into a new tube and perform the assay immediately. Dilute sample supernatant 1:10 with Furin Assay Buffer. Add 15 µl of diluted sample supernatant into a two wells of a 96 well white plate with flat bottom labeled as **Sample(s) without Furin Inhibitor [$S_{No\ In}$] & Sample(s) with Furin Inhibitor [S_{In}]**. Add 2 µl Deactivator into both wells. Bring the volume to 25 µl/well with Furin Assay Buffer. Prepare a **Background Control [BC]** well by adding 50 µl of Furin Assay Buffer into the desired well(s).

2. Furin Inhibitor Mix: Prepare enough Furin Inhibitor Mix for the number of assays to be performed. Prepare 25 µl Furin Inhibitor Mix per reaction as shown below.

	Furin Inhibitor Mix
Furin Assay Buffer	24 µl
Furin Inhibitor (100 µM)	1 µl

Add 25 µl of Furin Inhibitor Mix into the **[S_{In}]** well and 25 µl Furin Assay Buffer into the **[$S_{No\ In}$]** well respectively.

Note: For Unknown Samples, we suggest testing several dilutions of your sample to make sure the readings are within the AMC Standard Curve Range.

3. Furin Positive Control: Add 8 µl of diluted Furin Positive Control into the desired well(s) labeled as Positive Control **[PC]**. Adjust the volume to 50 µl/well by adding 42 µl Furin Assay Buffer. At this stage, all wells including Sample(s), Positive Control and Background Control contain 50 µl/well. Incubate the plate for 30 min at RT, protected from light.

4. AMC Standard Curve Preparation: Dilute AMC Standard to 100 µM AMC Standard by adding 10 µl of 1 mM AMC Standard to 90 µl of DMSO. Further dilute 100 µM AMC Standard to 10 µM (10 pmol/µl) with dH_2O and mix well. Add 0, 2, 4, 6, 8, 10, 12 and 14 µl of 10 µM AMC Standard into a series of wells in a 96-well white plate with flat bottom to generate 0, 20, 40, 60, 80, 100, 120 and 140 pmol/well of AMC Standard. Adjust the volume to 100 µl/well with Furin Assay Buffer. **Note:** Store the 100 µM AMC Standard at -20 °C.

5. Furin Substrate Mix Preparation: Mix enough Substrate Mix for the number of assays to be performed. Prepare 50 µl Substrate Mix per reaction.

	Substrate Mix
Furin Assay Buffer	49.8 µl
Furin Substrate	0.2 µl

Add 50 µl Substrate Mix to all wells including **[$S_{No\ In}$] & [S_{In}], [PC]** and **[BC]** 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. The AMC Standard Curve can be read in endpoint mode (i.e. during the incubation time).

6. Calculation: Subtract the 0 Standard RFU readings from all Standard RFU readings. Plot the AMC Standard Curve. For all Samples, choose any two time points within the linear range of the curve (t_1 & t_2). Calculate the net fluorescence signal (F) by subtracting the Background RFU Reading from all Sample(s), **[$S_{No\ In}$] & [S_{In}]**, and Furin Positive Control **[FC]** for the chosen t_1 & t_2 time points. Then obtain ΔRFU by subtracting from **[$S_{No\ In}$]** the **[S_{In}]**. Apply the corrected ΔRFU to AMC Standard Curve to get **B** pmol of AMC generated by Furin during the reaction time ($\Delta t = t_2 - t_1$).

Calculate the Furin activity in Samples using the following equation:

$$\textbf{Sample Furin Activity} = B/(\Delta t \times V) \times D = \text{pmol/min/µl} = \text{µU/µl} = \text{mU/ml}$$

Wher **B** is the amount of AMC calculated from the AMC Standard Curve (pmol)

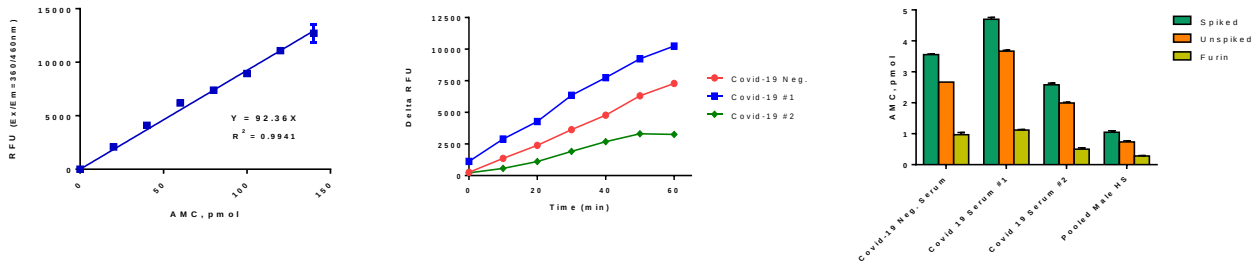
V is the volume of sample added to the well (µl)

Δ**T** is the time between t_2 and t_1 in min

D is the sample dilution factor (D= 1 for undiluted samples)

Unit Definition: One unit of Furin is the amount of enzymes that cleaves a fluorescent peptidyl substrate and liberates 1 µmol AMC per minute at pH 7.5, 25 ºC.

(a) **(b)** **(c)**



Figures: (a) AMC Standard Curve. (b) Kinetic activity of Furin Activity in human serum samples including one Covid-19 negative donor serum and two Covid-19 positive donor serums respectively. The results are presented as ΔRFU, fluorescence detected in the presence of the inhibitor is subtracted from the fluorescence detected in the absence of the inhibitor. (c) Furin Activity in human serum samples spiked with different amount of Furin Positive Control. The average spike recoveries were 90.94%, 92.05% and 114.78%, respectively. Assay was performed following the kit protocol.

VIII. Related Products:

Furin, Human Recombinant (Cat. # P1658)
Furin/PCSK3, Human CellExp™, hr (Cat. # 7249)
Furin/PACE Polyclonal Antibody (Cat. # 3873)
Furin/PACE Blocking Peptide (Cat. # 3873BP)
Recombinant Human beta-Secretase 1 (Cat. # 7609)
Furin Inhibitor Screening Kit (Fluorometric) (Cat. # K2069)
β-Secretase (BACE1) Activity Assay Kit II (F) (Cat. # K388)
β-Secretase (BACE1) Inhibitor Screening Kit (F) (Cat. # K720)
Human CellExp™ BACE1, human recombinant (Native) (Cat. # 7398)
Recombinant Ebolavirus BDBV Small/secreted Glycoprotein (Cat. # P1059)

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