ab308279 – Furin Inhibitor Screening Kit (Fluorometric)

Screening or characterizing Furin inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: Abcam website

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Furin Assay Buffer	25 ml	-
Furin Substrate	25 µl	-
Furin, Human Recombinant	40 µl	-20 °C
Furin Inhibitor (1 mM) (Avoid light)	25 µl	-20 °C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

Furin Inhibitor Screening Protocol:

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.

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.

Δ 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).

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_{50} 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.

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.

	Substrate Mix
Furin Assay Buffer	49.8 µl
Furin Substrate	0.2 µl

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.

Measurement:

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

Calculation:

Obtain ΔRFU 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 ΔRFU by time Δt (t_2 - t_1). If [SC] slope is significantly different from [EC] slope, use its values instead of EC in the calculations shown below.

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

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

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)