ab289844 – Furin Activity Assay Kit (Fluorometric)

For determining Furin activity in plasma, serum, and other samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab289844

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

On receipt unopened assay kit can be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Furin Assay Buffer	25 mL	-20°C
Furin Substrate	25 µL	-20°C
Furin Positive Control	10 µL	-20°C
Furin Inhibitor (1 mM)	25 µL	-20°C
Protease Deactivator/Deactivator (50 mM)	200 µL	-20°C
AMC Standard	100 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

- Before using the kit, spin the tubes prior to opening.

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.

Protease Deactivator/Deactivator (50 mM in DMSO): Warm to RT. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.

Sample Preparation

Serum & Plasma:
Store Serum & Plasma at -80°C to avoid loss of bioactivity and contamination. Samples to be used within 5 days may be stored at -20°C. Avoid multiple freeze-thaw cycles. Centrifuge sample(s) at 12,000 x g and 4°C for 10 min. Collect the supernatant into a new tube and perform the assay immediately.

Assay Protocol

1. Dilute sample supernatant 1:10 with Furin Assay Buffer.
2. For each of your samples, add 15 µL of diluted sample supernatant into two wells of a 96 well plate with a flat bottom, labeled as Sample(s) without Furin Inhibitor [$S_{No\ In}$] & Sample(s) with Furin Inhibitor [S_{In}].
3. Add 2 µL Protease Deactivator/Deactivator into both wells. Bring the volume to 25 µL/well with Furin Assay Buffer.
4. Prepare a Background Control [BC] well by adding 50 µL of Furin Assay Buffer into the desired well.

Furin Inhibitor Mix:

1. 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

2. 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.

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

Furin Positive Control:

1. Add 8 µL of diluted Furin Positive Control into the desired well(s) labeled as Positive Control [PC].
2. 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.
3. Incubate the plate for 30 min at RT, protected from light.

AMC Standard Curve Preparation:

1. Dilute AMC Standard to 100 µM by adding 10 µL of 1 mM AMC Standard to 90 µL of DMSO.
2. Further dilute the 100 µM AMC Standard to 10 µM (10 pmol/µL) with dH2O and mix well.
3. 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.
4. Adjust the volume to 100 µL/well with Furin Assay Buffer to generate a series of 0, 20, 40, 60, 80, 100, 120 and 140 pmol/well of AMC Standard.

ΔNote: *Store the 100 µM AMC Standard at -20°C.*

Furin Substrate Mix Preparation:

1. Mix enough Substrate Mix for the number of assays to be performed. Prepare 50 µL Substrate Mix per reaction.

	Furin Inhibitor Mix
Furin Assay Buffer	49.8 µL
Furin Substrate	0.2 µL

2. 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.

Measurement

Measure the fluorescence in kinetic mode at Ex/Em = 360/460 nm at 5 min intervals for 30-60 mins at RT. The AMC Standard Curve can be read in endpoint mode (i.e. during the incubation time).

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 [S_{In}] from [$S_{No\ In}$]. Apply the corrected ΔRFU to AMC Standard Curve to get B pmol of AMC generated by Furin during the reaction time ($\Delta t = t_2 - t_1$).

Sample Furin Activity = $B/(\Delta t \times V) \times D$ = pmol/min/µL = µU/µL = mU/mL

Where:
B = the amount of AMC calculated from the AMC Standard Curve (pmol)
Δ**t** = the time between t_2 and t_1 in min
V = the volume of sample added to the well (µL)
D = Sample dilution factor (D= 1 for undiluted samples)

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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)