Data Sheet

Furin Protease Assay Kit

Catalog #AMS.78040

Size: 96 reactions

BACKGROUND: Furin is a member of the proprotein convertase (PC) family, which belongs to the subtilisin superfamily of serine protease. This protease is thought to cleave and activate more than 150 mammalian, viral and bacterial substrates. Among them are viral envelope glycoproteins and bacterial toxins, as well as cellular factors that can promote tumor development and growth. Cleavage of the SARS coronavirus spike glycoprotein by Furin is a critical step in viral fusion and entry, making furin a potential target for SARS-CoV-2 therapy.

DESCRIPTION: The *Furin Protease Assay Kit* is designed to measure Furin Protease activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The kit comes in a convenient 96-well format, with purified Furin, fluorogenic substrate, and Furin assay buffer for 100 enzyme reactions. The Furin inhibitor Chloromethylketone is also included as a positive control.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	Recombinant Furin	3 µg	-80°C	***Avoid freeze/ thaw cycles!***
	Furin Protease Substrate (500 µM)	50 µl	-80°C	
	Furin Assay Buffer	25 ml	-20°C	
78713	Chloromethylketone (100 µM)	20 µl	-80°C	
79685	Black, low binding microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading λexc/λem=380 nm/460 nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: At least one year from date of receipt when stored as directed.

REFERENCE(S):

1. Braun, E. and Sauter, D. (2019). Furin-mediated protein processing in infectious diseases and cancer. *Clin Transl Immunol*, **8:** e1073. doi:10.1002/cti2.1073**.**
2. Follis, K.E., *et al.* (2006). Furin cleavage of the SARS coronavirus spike glycoprotein enhances cell-cell fusion but does not affect virion entry. *Virology*. **350(2):**358-369. doi:10.1016/j.virol.2006.02.003

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **Furin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot **Furin** into single use aliquots. Single-use aliquots should have no less than 2 µl. Store remaining undiluted enzyme in aliquots at -80°C. Note: **Furin** enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.
2) Dilute **Furin** in **Assay buffer** at 0.5 ng/µl (25 ng per reaction).
3) Add 50 µl **diluted Furin** enzyme solution to wells designated as "Positive Control," "Inhibitor Control," and "Test Sample." Add 50 µl **Assay buffer** to the "Blank" wells.

Component	Positive Control	Test Sample	Inhibitor Control	Blank
Furin (0.5 ng/µl)	50 µl	50 µl	50 µl	–
Assay Buffer	–	–	–	50 µl
Chloromethylketone (0.5 µM)	–	–	10 µl	–
Test Inhibitor	–	10 µl	–	–
Inhibitor Buffer	10 µl	–	–	10 µl
Substrate solution	40 µl	40 µl	40 µl	40 µl
Total	**100 µl**	**100 µl**	**100 µl**	**100 µl**

4) Dilute 100 µM **Chlormethylketone** 200-fold in **Assay Buffer** to obtain a 0.5 µM solution. Add 10 µl **Chloromethylketone** (0.5 µM) to the wells labeled "Inhibitor Control."
5) Prepare the inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step the compound concentration is 10-fold higher than the final concentration).

   If the inhibitor compound is dissolved in 1x buffer, make a solution of the compound 10-fold higher than the final concentration in Furin assay buffer. For example, diluting 2 µl of 100 µM Chloromethylketone in 398 µl Assay Buffer (step 4) creates a 0.5 µM solution. Adding 10 ul to the assay (final volume 100 µl) results in a 0.05 µM final concentration.

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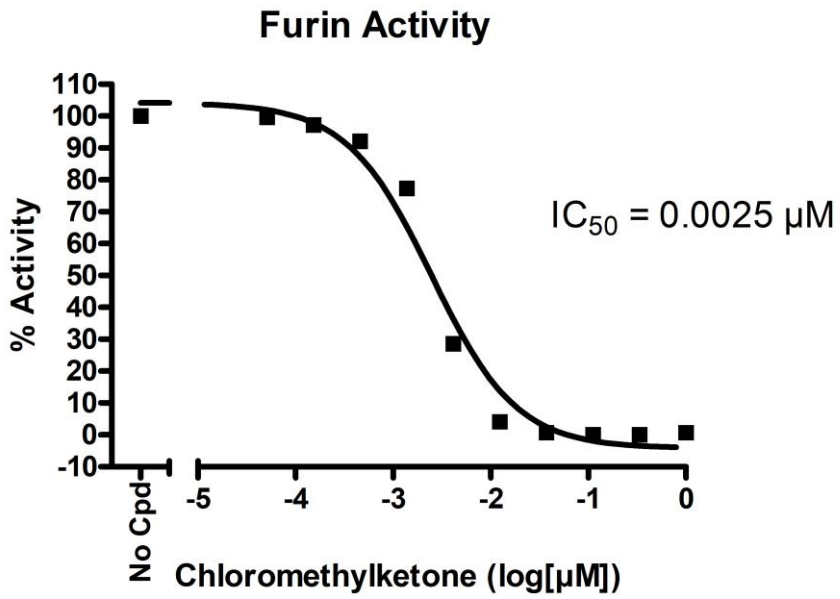
6) Add 10 µl test inhibitor to each well designated “Test Sample.” Add 10 µl Inhibitor Buffer (either 1X assay buffer or 10% DMSO in 1X assay buffer, depending on which inhibitor solution is used) to “Blank” and “Positive Control” wells.

7) Dilute 500 µM **Furin protease substrate** 1:100 in assay buffer, to make a 5 µM solution. Dilute only enough as is required for the assay.

8) Start reaction by adding 40 µl of the substrate solution to each well (Final concentration of the **Furin protease substrate** in a 100 µl reaction is 2 µM).

9) Incubate at room temperature for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at wavelength 380 nm and detection of emission at wavelength 460 nm. The fluorescence intensity can also be measured kinetically. “Blank” value is subtracted from all other values.

EXAMPLE OF ASSAY RESULTS:



Inhibition of Furin enzyme activity by Chloromethylketone, measured using the *Fluorogenic Furin Protease Assay Kit (#78040).* Fluorescence intensitywas measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. Forlot-specific information, please contact AMS Biotechnology at info@amsbio.com*

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RELATED PRODUCTS

Product	Cat. #	Size
3CL Protease Assay Kit	79955	96/384 rxns
Papain-like Protease (SARS-CoV-2) Assay Kit	79995	96 reactions
Recombinant 3CL Protease, MBP-tag	100707-1	100 µg
PLPro, His-tag (SARS-CoV-2)	100735	20 µg/50 µg
PLPro, His-tag (SARS-CoV)	81091	25 µg
SARS-CoV-2 Spike:ACE2 Inhibitor Screening Kit	79931	96 reactions
ACE2:SARS-CoV-2 Spike Inhibitor Screening Kit	79936	96 reactions
ACE2:SARS-CoV-2 Spike S1-Biotin Inhibitor Screening Kit	79945	96 reactions
SARS-CoV-2 Spike S1-Biotin:ACE2 TR-FRET Kit	79949	96 reactions
Spike S1, Fc Fusion, Avi-tag (SARS-CoV-2)	100678	100 µg/1 mg
Spike S1, Fc fusion, Avi-tag, Biotin-Labeled	100679	25 µg/50 µg
Spike S1 RBD, His-tag (SARS-CoV-2)	100687	50 µg/100 µg
Spike S1, Fc fusion (SARS-CoV-2)	100688	20 µg/50 µg
Spike S1 RBD, Fc fusion (SARS-CoV-2)	100699	50 µg/100 µg
ACE2 Inhibitor Screening Assay Kit	79923	96 reactions
ACE2, His-tag	11003	20 µg/100 µg
ACE2, His-Avi-Tag, Biotin-labeled HiP™	100665	20 µg/50 µg

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