

## Data Sheet

### ***Furin Protease Assay Kit***

**Catalog #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               | <b>Avoid<br/>freeze/<br/>thaw<br/>cycles!</b> |
|           | Furin Protease Substrate (500 µM)   | 50 µl  | -80°C               |   |
|           | Furin Assay Buffer                  | 25 ml  | -20°C               |   |
|           | Chloromethylketone (100 µM)         | 20 µl  | -80°C               |   |
| 79685     | Black, low binding microtiter plate | 1      | Room<br>Temperature |   |

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

Fluorescent microplate reader capable of reading  $\lambda_{exc}/\lambda_{em}=380\text{ nm}/460\text{ nm}$

**APPLICATIONS:** Great for studying enzyme kinetics and HTS applications.

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

**REFERENCE(S):**

- 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](https://doi.org/10.1002/cti2.1073).
- 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. 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 (no inhibitor) | 10 μl            | –             | –                 | 10 μl         |
| Substrate solution              | 40 μl            | 40 μl         | 40 μl             | 40 μl         |
| <b>Total</b>                    | <b>100 μl</b>    | <b>100 μl</b> | <b>100 μl</b>     | <b>100 μl</b> |

- 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 water, 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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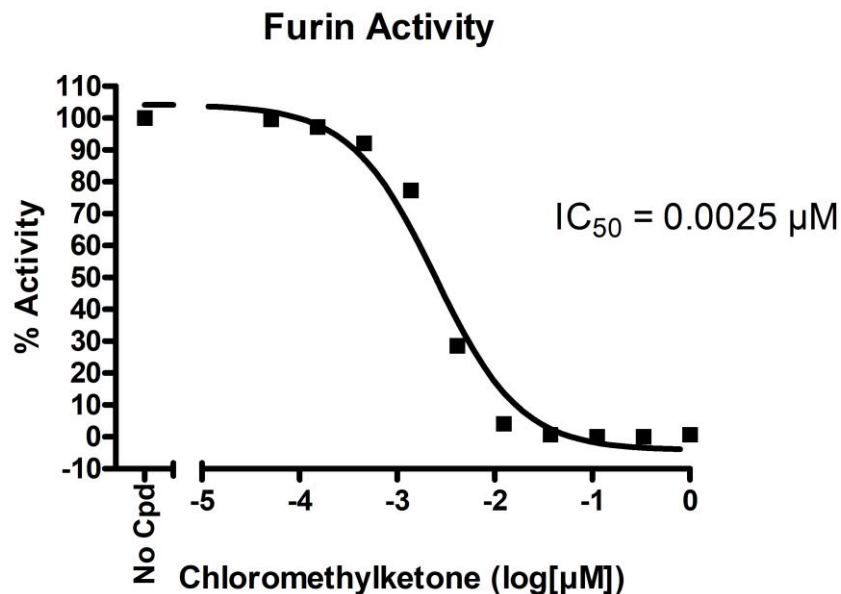
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- 6) Add 10  $\mu$ l test inhibitor to each well designated "Test Sample". Add 10  $\mu$ l 1X assay buffer or 10% DMSO (depending on which inhibitor solution is used) to "Blank" and "Positive Control" wells.
- 7) Dilute 500  $\mu$ M **Furin protease substrate** 1:100 in assay buffer, to make a 5  $\mu$ M solution. Dilute only enough as is required for the assay.
- 8) Start reaction by adding 40  $\mu$ l of the substrate solution to each well (Final concentration of the **Furin protease substrate** in a 100  $\mu$ l reaction is 2  $\mu$ M).
- 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 (BPS Bioscience #78040)*. Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)*

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**RELATED PRODUCTS**

| <b><u>Product</u></b>                                   | <b><u>Cat. #</u></b> | <b><u>Size</u></b> |
|---|----------------------|--------------------|
| 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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