Description

The Trypsin-2 Activity Assay Kit is a colorimetric assay kit designed to measure trypsin-2 protease activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant trypsin-2, substrate, and assay buffer for 100 enzyme reactions.

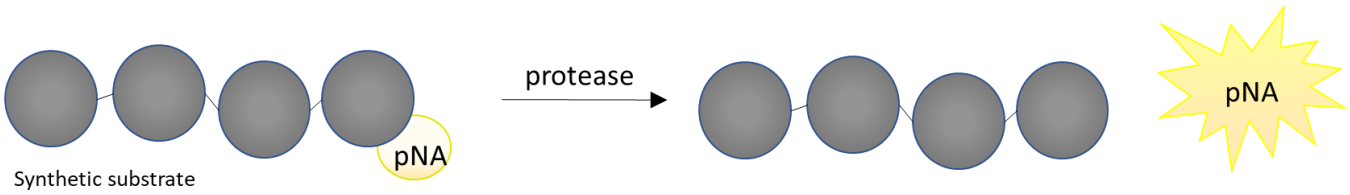

Figure 1: Illustration of the assay principle.

Upon proteolysis, trypsin-2 cleaves the chromogenic substrate at the C-terminal end releasing p-nitroanilid (pNA), which produces a yellow color that is measurable photometrically at λ=405 nm. The increase in color is proportional to trypsin-2 activity.

Background

Trypsin is a serine protease from the pancreas, that hydrolyzes peptides specifically at the carboxyl side of arginine and lysine residues. It is formed as a proenzyme (trypsinogen) in the pancreas and secreted to the duodenum when the pancreas is activated by cholecystokinin. It is then converted to its active form in the digestive system by enterokinase, where it supports digestion by degrading proteins into smaller peptides. Trypsin has multiple applications in biotechnology, being commonly used in tissue culture to resuspend adherent cells, dissociate cells from tissues, generate peptides for proteomic studies and in multiple food related processes. Early activation of trypsin in the pancreas can result in pancreatitis. Trypsin is also involved in SARS-CoV2 (severe acute respiratory syndrome coronavirus 2), by enabling viral entry. Trypsin inhibitors are present in several foods, such as soybeans, as a mechanism of defense against digestion, and protect animals from self-digesting the pancreas. TATI (peptide-tumor-associated trypsin inhibitor) is a marker of late stage ovarian, gastric and pancreatic cancer and it is found at high levels in kidney failure patients. The use of trypsin inhibitors as antibacterial and anti-viral agents in the food industry and as therapy has been under investigation and may offer a promising approach as bactericides.

Applications

Screen small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	Human Trypsin-2 (1 µg/µl)	10 µg	-80°C
	PR Substrate 3	1 ml	-80°C
	PR-02 Buffer	10 ml	-20°C
79963	96-well clear microplate	1	Room Temp

Materials Required but Not Supplied

- Spectrophotometer capable of measuring absorbance at λ= 405 nm.
- Adjustable micropipettor and sterile tips

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Negative Control", "Positive Control" and "Test inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using the inhibitor nafamostat mesylate or soybean trypsin inhibitor as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw Trypsin-2, on ice. Briefly spin the tubes to recover the full content.

2. Dilute Trypsin-2 to 2.5 ng/µl with PR-02 Buffer (40 µl/well).

3. Prepare the Test Inhibitor (10 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 100 µl.

   3.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations using PR-02 Buffer.

   For the positive and negative controls, use PR-02 Buffer (Diluent Solution).

   OR

   3.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in PR-02 Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor in PR-02 Buffer at 10-fold the desired final concentrations using 10% DMSO in to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

4. Add 40 µl of diluted Trypsin-2 to all wells, except "Negative Control" wells.

5. Add 10 µl of inhibitor solution to each well designated "Test Inhibitor".

6. Add 10 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.

7. Pre-incubate the Test inhibitor with the diluted Trypsin-2 for 30 minutes at Room Temperature (RT) with gentle agitation.

8. Dilute 5-fold the PR Substrate 3 with PR-02 Buffer (50 µl/well).

9. Initiate the reaction by adding 50 µl of the diluted Substrate to all wells.

10. Incubate at RT for 60-90 minutes or perform kinetic analysis.

Component	**Negative Control**	**Positive Control**	**Test Inhibitor**
PR-02 Buffer	40 µl	-	-
Test inhibitor	-	-	10 µl
Diluent Solution	10 µl	10 µl	-
Diluted Trypsin-2 (2.5 ng/µl)	-	40 µl	40 µl
30 minutes of pre-incubation at Room Temperature			
Diluted PR Substrate 3 (5-fold)	50 µl	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

11. Measure absorbance at λ=405 nm. Subtract "Blank" value from all other values. If compounds absorb at 405 nm it is recommended to read the plate at time 0 as well as the final timepoint. The time 0 measurement can be subtracted from the final reading to account for compound absorbance.

Example Results

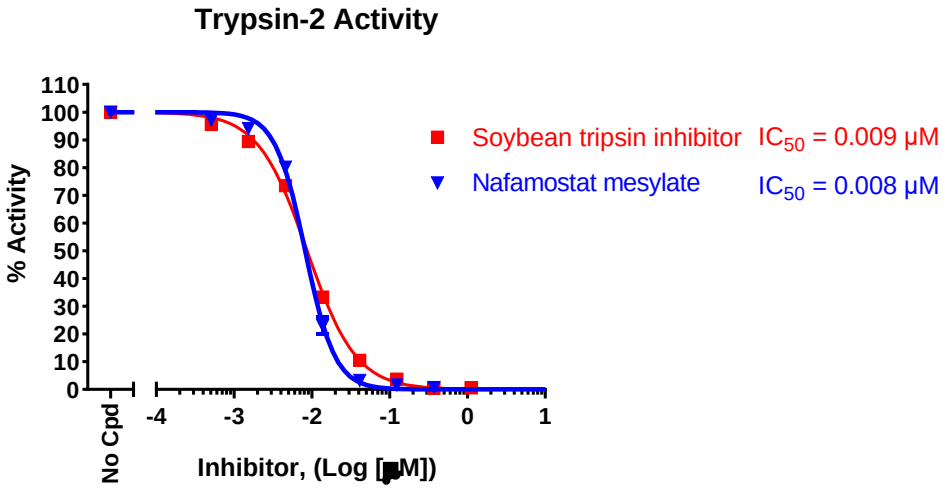

Figure 1. Human Trypsin-2 activity is inhibited by soybean trypsin inhibitor and nafamostat mesylate.
Trypsin-2 activity was measured in the presence of increasing concentrations of soybean trypsin inhibitor (MedChem #HY-126388) or Nafamostat mesylate (MedChem #HY-B0190A).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Kim Y., *et al.*, 2022 *Arch Virol.* 167(2): 441-458.
Nascimento A., *et al.*, 2022 *J Enzyme Inhib Med Chem* 37(1):749-759.

Related Products

Products	*Catalog #*	*Size*
Neutrophil Elastase Inhibitor Screening Assay Kit	82090	96 reactions/384 reactions
ELANE (Elastase), Avi-His-Tag HiP™ Recombinant	101141	25 µg/100 µg
ELANE (Elastase), Avi-His-Tag, Biotin Labeled HiP™ Recombinant	101142	25 µg/50 µg

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