

Description

The MASP2 Fluorogenic Assay Kit is designed to measure the activity of MASP2 (Mannan-binding lectin–associated serine proteinase 2) for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant MASP2 (amino acids 288-686), fluorogenic substrate and assay buffer for 100 enzyme reactions.

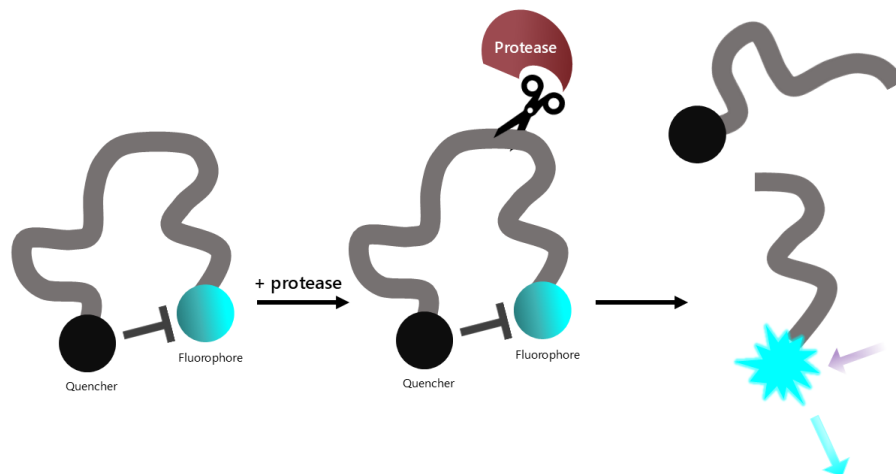


Figure 1. Mechanism of action of MASP2 Fluorogenic Assay Kit.

MASP2 is incubated with a fluorogenic substrate which is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent substrate from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

MASP2 (mannan-binding lectin–associated serine proteinase 2) is a serine protease that is the central effector enzyme of the lectin pathway of the complement system. Although this pathway plays an important role in innate antimicrobial defense, its overactivation contributes to ischemia-reperfusion injury and a range of immune-mediated and inflammatory disorders. Experimental studies in mice demonstrated that selective targeting of MASP2 effectively inhibits the lectin pathway while preserving the classical and alternative pathways, thereby maintaining core complement-mediated host defense. Therapies targeting MASP2 are being developed, such as a first-in-class N-acetylgalactosamine-conjugated small interfering RNA (siRNA). FB7013, one such therapeutic, inhibits the lectin pathway during immunoglobulin A nephropathy (IgAN). Another strategy involves the use of recombinant antibodies, such as Narsoplimab, also known as Yartemlea, which has been approved for the treatment of infections. Further studies will open new therapeutic avenues for the treatment of MASP2-related disorders.

Applications

Screen small molecule inhibitors or antagonists that affect activity of MASP2 in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102704-KC5.5	MASP2, His-Tag*	5.5 µg	-80°C
84204-KC100	50x Fluorogenic MASP2 Substrate (Lyophilized)	100 µg	-80°C
84405-KC2	5x MASP2 Assay Buffer	2 ml	-20°C
79685	96-well black, low binding microplate	1	Room Temp

*The concentration of the protein is lot-specific and will be indicated on the tube.

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Fluorescent microplate reader capable of reading $\lambda_{exc}/\lambda_{em}$ = 320 nm/420 nm
- Orbital shaker

Storage Conditions

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.

Contraindications

- The final concentration of DMSO in the reaction should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples should be run in duplicate.
- The assay should include “Blank”, “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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
- We recommend using Human Serpin G1 (AcroBiosystems, #SER-H52H5) as internal controls for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X and 10X the IC₅₀ value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com/serial-dilution-protocol).

1. Thaw **MASP2** and **50x Fluorogenic MASP2 Substrate (Lyophilized)** on ice. Briefly spin tube to recover its full contents.
2. Thaw **5x MASP2 Assay Buffer** at Room Temperature (RT).
3. Prepare **1x Assay Buffer** by diluting 5x MASP2 Assay Buffer 5-fold with distilled water.
4. Dilute **MASP2** to 2.75 ng/μl with 1x Assay Buffer (20 μl/well).
5. Add 20 μl of **diluted MASP2** to the “Positive Control” and “Test Inhibitor” wells.
6. Add 20 μl of **1x Assay Buffer** to the “Blank” wells.
7. Prepare the **Test Inhibitor** (5 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 50 μl.

7.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations using the 1x Assay Buffer.

For positive and negative controls use 1x Assay Buffer (Diluent Solution).

OR

7.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO, at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate serial dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

8. Add 5 μl of the **Test Inhibitor** solution to each well designated as “Test Inhibitor”.
9. Add 5 μl of **Diluent Solution** to the “Positive Control” and “Blank” wells.
10. Pre-incubate at RT for 30 minutes with gentle agitation.
11. Add 150 μl of distilled water to the **50x Fluorogenic MASP2 Substrate (Lyophilized)** vial to dissolve the content.

- Prepare **2x Fluorogenic MASP2 Substrate** by diluting **50x Fluorogenic MASP2 Substrate** 25-fold with 1x Assay Buffer (25 µl/well).
- Add 25 µl of 2x Fluorogenic MASP2 Substrate to all wells. Protect your samples from direct exposure to light.
- Incubate at RT for 75 minutes with gentle agitation or perform kinetic analysis.

	Blank	Positive Control	Test Inhibitor
Diluted MASP2 (2.75 ng/µl)	-	20 µl	20 µl
1x Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Pre-incubate 30 minutes at Room Temperature			
2x Fluorogenic MASP2 Substrate	25 µl	25 µl	25 µl
Total	50 µl	50 µl	50 µl

- Read the fluorescence intensity of the samples ($\lambda_{\text{excitation}} = 320 \text{ nm}$; $\lambda_{\text{emission}} = 420 \text{ nm}$ with bandwidth 10 nm) in an appropriate microplate reader.
- The “Blank” value should be subtracted from all other values.

Example Results

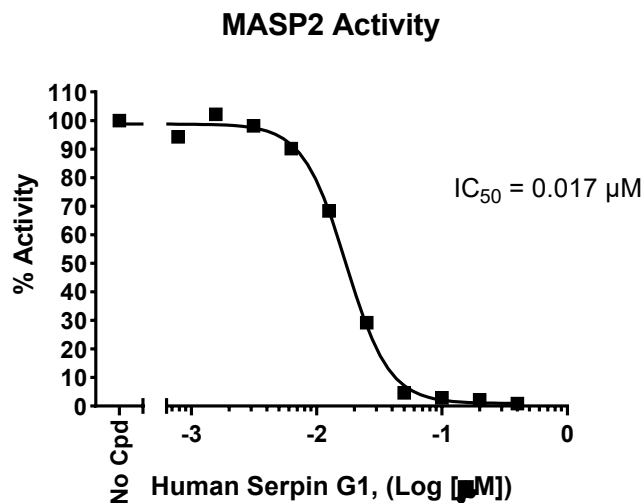


Figure 2: Inhibition of MASP2 activity by the inhibitor Human Serpin G1. MASP2 activity was measured in the presence of increasing concentrations of Human Serpin G1. The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (enzyme activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

References

- Szakács D., *et al.*, 2019 *J Biol Chem* 294(20): 8227-8237.
Schwaeble W. J., *et al.*, 2011 *Proc Natl Acad Sci U.S.A.* 108 (18): 7523-8.
Fu J. *et al.*, 2016 *Microb Pathog* 100: 221-28.
Garcia-Laorden M. I. *et al.*, 2020 *J Clin Immunol* 40 (1): 203-10.

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