Description

The ADAMTS4 Fluorogenic Assay Kit is designed to measure ADAMTS4 (A disintegrin and a metalloproteinase with thrombospondin motifs 4) protease activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant ADAMTS4, fluorogenic substrate, and assay buffer for 100 enzyme reactions.

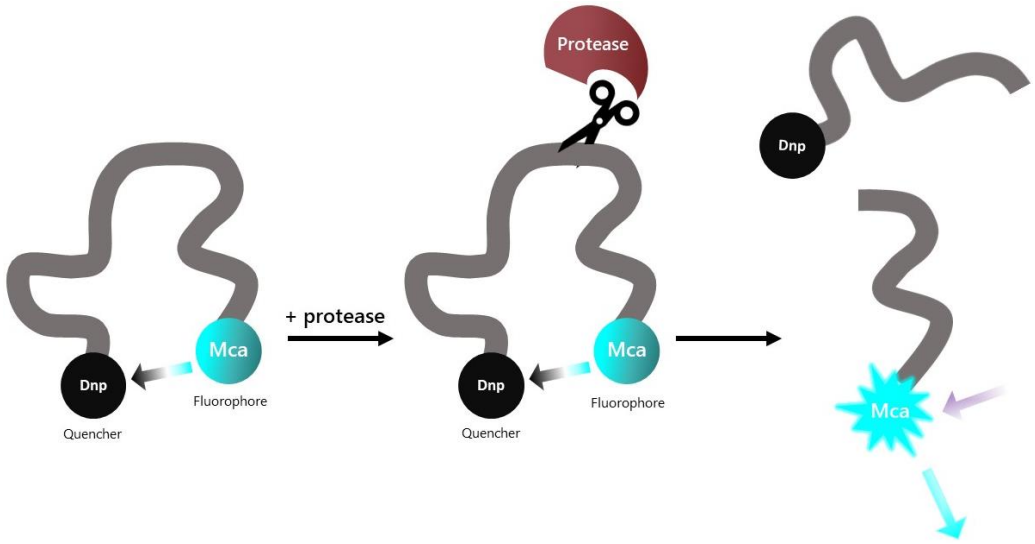

Figure 1: Illustration of the mechanism behind the ADAMTS4 Fluorogenic Assay Kit.
ADAMTS4 is incubated with a fluorogenic substrate which is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent Mca from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

ADAMTS4 (A disintegrin and metalloproteinase with thrombospondin motifs 4), also known as aggrecanase-1, is a member of the ADAMTS proteinase family but lacks the C-terminal domain and it is the only member of the family that does not undergo glycosylation. Its TSR (thrombospondin type 1 motif) is essential for its function, by binding to the extracellular matrix proteins. ADAMTS4 can be found in several tissues, such as the ovary, retina, breast, brain and others, and it is involved in cleaving CSPGs (chondroitin sulfate hyaluronan-binding proteoglycans), such as aggrecan, neurocan and versican. It can be inhibited by TIMP3 (metalloproteinase inhibitor 3), and dysfunction can result in osteoarthritis. In addition, it can be a biomarker for adult cardiac injury, as it becomes expressed under those conditions and contributes to fibrosis. In a rat model, inhibition of ADAMST4 resulted in improved cardiac responses and lower accumulation of collagen. The development of ADAMST4 inhibitors can thus open new therapeutic avenues in cardiology.

Applications

Study enzyme kinetics and screen small molecule inhibitors of ADAMTS4 for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	ADAMTS4*	20 µg	-80°C
	ADAMTS Fluorogenic Substrate	50 µg	-80°C
	ADAMTS Assay Buffer	5 ml	-20°C
79685	Low binding, black 96-well plate	1	Room Temperature

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

Materials Required but Not Supplied

- Fluorimeter capable of excitation at λ=330 nm (15 nm bandwidth) and detection at λ=430 nm (10 nm bandwidth)
- Protease Free Water
- Adjustable micropipettor and sterile tips
- 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 assay 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.
- The presence of strong acids or bases, ionic detergents and high salt should be avoided.

Assay Protocol

- All samples and controls should be performed 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).
- We recommend using ADAMTS-5-IN-3 (MedChem Express #HY-145061) 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.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **ADAMTS Assay Buffer.**

2. Thaw **ADAMTS4** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

3. Dilute ADAMTS4 to 10 ng/µl with ADAMTS Assay Buffer (20 µl/well). For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

4. 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.

   4.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in ADAMTS Assay Buffer.

   For the positive and negative controls, use ADAMTS Assay Buffer (Diluent Solution).

   OR

   4.2 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 ADAMTS Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using ADAMTS Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in ADAMTS 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%.

5. Add 20 µl of diluted ADAMTS4 to the “Positive Control” and “Test Inhibitor” wells.

6. Add 20 µl of ADAMTS Assay Buffer to the “Blank” wells.

7. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

8. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

9. Incubate at Room Temperature (RT) for 30 minutes.

10. Dissolve ADAMTS Fluorogenic Substrate with 30 µl of protease free water. This makes **1 mM ADAMTS Fluorogenic Substrate**.

    Note: Reconstituted ADAMTS Fluorogenic Substrate can be stored into single use aliquots (minimum volume 5 µl/aliquot) at -80°C.

11. Dilute 1 mM ADAMTS Fluorogenic Substrate 100-fold with ADAMTS Assay Buffer (25 µl/well).

12. Add 25 µl of diluted ADAMTS Fluorogenic Substrate to every well.

13. Incubate at RT for 4 hours with gentle agitation.

Component	Blank	Positive Control	Test Inhibitor
Diluted ADAMTS4 (10 ng/µl)	-	20 µl	20 µl
ADAMTS Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
30 minutes at Room Temperature			
Diluted ADAMTS Fluorogenic Substrate (10 µM)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

14. Read the plate in a fluorimeter capable of excitation at λ=330 nm (15 nm bandwidth) and detection at λ=430 nm (10 nm bandwidth).

15. The "Blank" value should be subtracted from all other readings.

Example Results

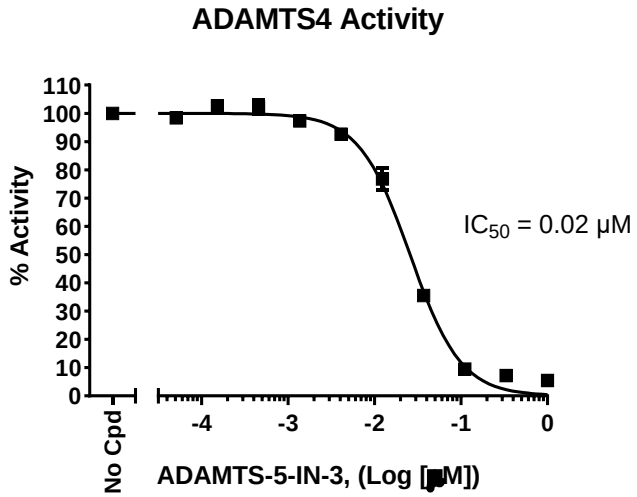

Figure 2: Inhibition of ADAMTS4 activity by the inhibitor ADAMTS-5-IN-3.
ADAMTS4 activity was measured in the presence of increasing concentrations of ADAMTS-5-IN-3 (MedChem Express #HY-145061). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

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

Vistnes M., *et al.*, 2023 *Cardiovasc Res* 119(10):1915-1927.
Khanan R., *et al.*, 2022 *Sci Rep* 12:9898.

Related Products

Products	*Catalog #*	*Size*
ADAM17 Fluorogenic Assay Kit	78000	96 reactions
ADAM10 Fluorogenic Assay Kit	78007	96 reactions
ADAM9 Fluorogenic Assay Kit	82537	96 reactions

Version 090624