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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The ADAM9 Fluorogenic Assay Kit is designed to measure ADAM9 (A disintegrin and a metalloprotease 9) protease activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant ADAM9, fluorogenic substrate and solution, and assay buffer for 100 enzyme reactions.

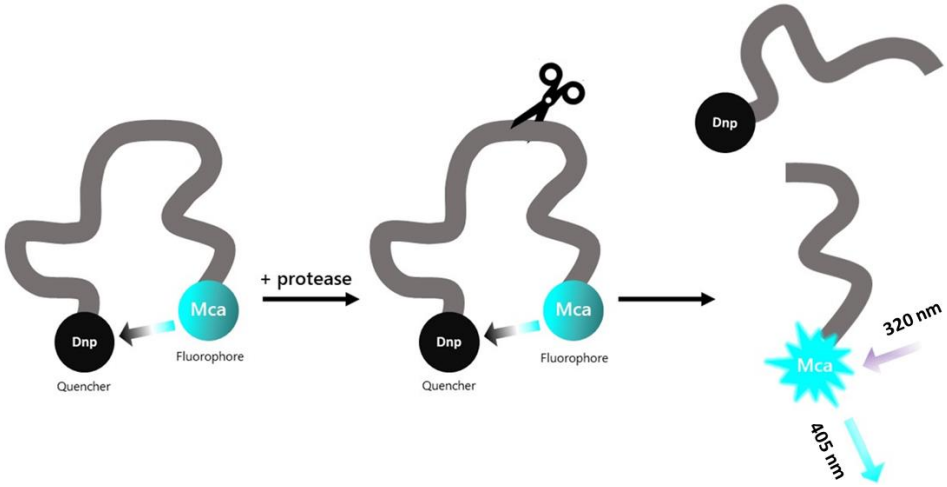

Figure 1: Illustration of the mechanism behind the ADAM9 Fluorogenic Assay Kit.
ADAM9 is incubated with a fluorogenic substrate which is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent Mca from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

Background

ADAM9 (A disintegrin and a metalloprotease 9), also known as MDC9 or meltrin-γ, is one of the 22 members of the ADAM family of proteins found in humans and part of the zinc protease superfamily. It has two main known functions, acting as a metalloprotease and in adhesion. It is involved in myogenesis, cell migration and proliferation, immune responses and cell interaction with other cells or matrixes. It can be found in monocytes, macrophages, neutrophils, keratinocytes, and fibroblasts. It acts on inflammatory responses by participating in monocyte fusion and the formation of MGCs (multinucleated giant cells). ADAM9 is found at high levels in many cancers, such as breast cancer, NSCLC (non-small cell lung cancer), prostate and cervical cancer, amongst others., and correlates with a poor prognosis. Its restricted expression in normal tissues makes it an attractive target in ADC (antibody -drug conjugate) therapy. In pre-clinical studies a maytansinoid-based ADC compound, IMGG936, showed a good pharmacokinetic profile and antitumor activity in CDX (cell line-derived xenograft) and PDX (patient-derived xenograft) models. Other strategies targeting ADAM9 involve the use of miRNA, the use of competitive proteins such as the mouse prodomain of ADAM9, and antibodies. In addition, ADAM9 has been linked to COPD (chronic obstructive pulmonary disease), vascular and neurodegenerative disorders. The development of therapies targeting this protein will no doubt result in advances in ADAM-9 related diseases.

Applications

Study enzyme kinetics and screen small molecule inhibitors of ADAM9 for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	ADAM9, His-Tag*	5 µg	-80°C
	ADAM Fluorogenic Substrate II	53 µg	-80°C
78001	1x ADAM 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=320 nm (5 nm bandwidth) and detection at λ=405 nm (5 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 on 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 TAPI-1 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 **1x ADAM Assay Buffer.**

2. Thaw ADAM9 on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

3. Dilute ADAM9 to 2.5 ng/µl with 1x ADAM 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 1x ADAM Assay Buffer.

   For the positive and negative controls, use 1x ADAM 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 1x ADAM Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using 1x ADAM 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 1x ADAM 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 ADAM9 to the “Positive Control” and “Test Inhibitor” wells.

6. Add 20 µl of 1x ADAM 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 ADAM Fluorogenic Substrate II with 30 µl of protease free water. This makes **1 mM ADAM Fluorogenic Substrate II**.

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

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

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

13. Protect your samples from direct exposure to light and incubate at room temperature for 4h with gentle agitation or perform kinetic analysis.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Diluted ADAM9 (2.5 ng/µl)	-	20 µl	20 µl
1x ADAM Assay Buffer	20 µl	-	-
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
30 minutes at Room Temperature			
Diluted ADAM Fluorogenic Substrate II (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=320 nm (5 nm bandwidth) and detection at λ=405 nm (5 nm bandwidth).

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

Example Results

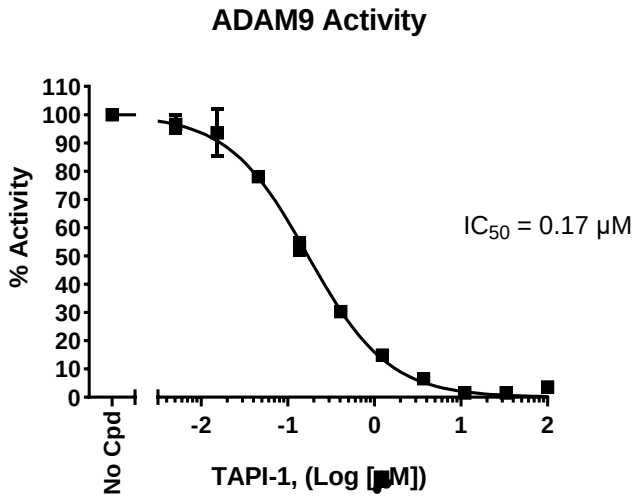

Figure 2: Inhibition of ADAM9 activity by the inhibitor TAPI-1.
ADAM9 activity was measured in the presence of increasing concentrations of TAPI-1 (Cayman Chemical #18505). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence 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
Scribner J., *et al.*, 2022 *Mol Cancer Ther* 21(7):1047:1059.
Chou C., *et al.*, 2020 *Int J Mol Sci* 21(20):7790.

Related Products

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

Version 111524