**Description**

The ADAM17 Fluorogenic Assay Kit is designed to measure ADAM17 (A disintegrin and a metalloprotease 17) protease activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough recombinant ADAM17, fluorogenic substrate and solution, and assay buffer for 100 enzyme reactions.

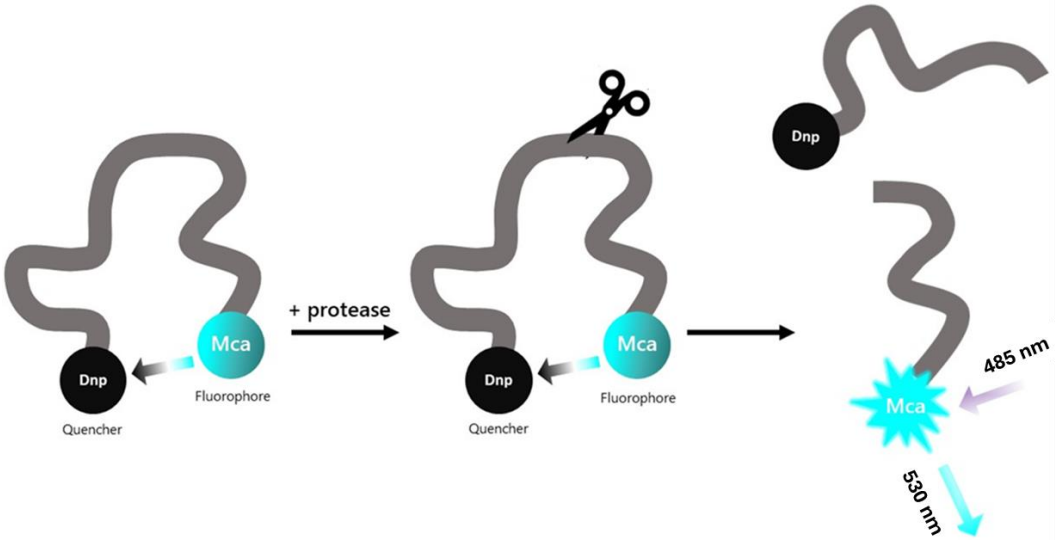


*Figure 1: Illustration of the mechanism behind the ADAM17 Fluorogenic Assay Kit.*
ADAM17 is incubated with a fluorogenic substrate which is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent Mca from the quencher. Fluorescence intensity increases proportionally to the activity of the protease.

**Background**

ADAM17 (A disintegrin and a metalloprotease 17), also known as TACE (TNF-α converting enzyme), is one of the 22 members of the ADAM family of proteins found in humans and part of the zinc protease superfamily. It is involved in development, immunity, inflammation and tumorigenesis. ADAM17 is found in the brain, heart, kidney and skeletal muscle, amongst others. It has more than 80 substrates, ranging from EGFR (epidermal growth factor receptor) ligands, TNF-α (tumor necrosis factor alpha) to adhesion molecules and amyloid precursor protein, and it is thus involved in multiple pathways. Its activity is highly regulated, with low activity resulting in issues with regeneration due to low EFGR signaling, while increased activity can lead to tumor development. This protein is formed in the ER (endoplasmic reticulum) as a proform and matures in the Golgi apparatus where the prodomain is cleaved by pro-protein convertases. Patients with RA (rheumatoid arthritis) have elevated activity of ADAM17 in the synovial tissue. Because of the roles it plays, targeting this protein in diseased tissues without affecting the healthy tissues is a major concern. The development of therapies targeting this protein in specific tissues will no doubt result in advances in ADAM-17 related diseases.

**Applications**

Study enzyme kinetics and screen small molecule inhibitors of ADAM17 for drug discovery and high throughput screening (HTS) applications.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| | ADAM17* | 3 µg | -80°C |
| | ADAM Fluorogenic Substrate I | 50 µl | -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=485 nm (5 nm bandwidth) and detection at λ=530 nm (5 nm bandwidth)
- 37°C incubator
- 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%.
- Fluorescent compounds that have λex=485 nm (5 nm bandwidth) and detection at λem=530 nm (5 nm bandwidth) can interfere with the readouts.
- 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** and **ADAM Fluorogenic Substrate I.**

2. Thaw ADAM17 on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

3. Dilute ADAM17 to 1.25 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 ADAM17 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. Dilute **ADAM Fluorogenic Substrate I** 50-fold with 1x ADAM Assay Buffer (25 µl/well).

11. Start the reaction by adding 25 µl of diluted ADAM Fluorogenic Substrate I to each well. Protect your samples from direct exposure to light and incubate at room temperature for 30 minutes or perform kinetic analysis.

| Component | Blank | Positive Control | Test Inhibitor |
|---|---|---|---|
| Diluted ADAM17 (1.25 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 I | 25 µl | 25 µl | 25 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** |

12. Read the plate in a fluorimeter capable of excitation at λ=485 nm (5 nm bandwidth) and detection at λ=530 nm (5 nm bandwidth).

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

**Example Results**

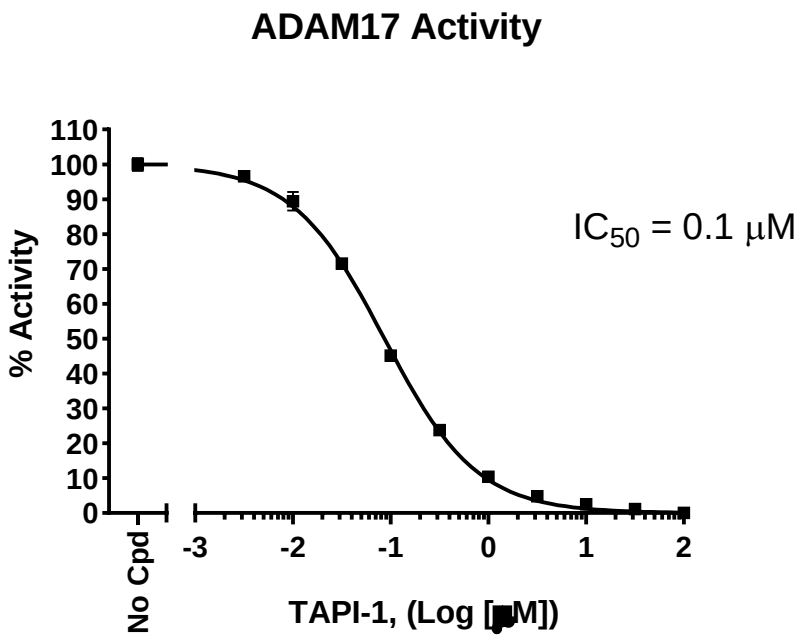


*Figure 2: Inhibition of ADAM17 activity by the inhibitor TAPI-1.*
ADAM17 activity was measured in the presence of increasing concentrations of TAPI-1 (Cayman Chemical #18505). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence 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**

Scheller Jürgen, *et al.*, 2011 *Trends in Immunology* 32(8): 380-387.
Blaydon D. C., *et al.*, 2011 *New England Journal of Medicine* 365(16): 1502-1508.
Gooz M., 2010 *Crit Rev Biochem Mol Biol* 45 (2):146-169.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| ADAM9 Fluorogenic Assay Kit | 82537 | 96 reactions |
| ADAM10 Fluorogenic Assay Kit | 78007 | 96 reactions |

*Version 111524*