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Data Sheet
ADAM17 Fluorescent Assay Kit
Catalog #78000
Size: 96 reactions

BACKGROUND: ADAM17 (ADAM Metallopeptidase Domain 17) is part of the ADAM family of disintegrins and metalloproteases. Initially identified as TNF- α converting enzyme (TACE), ADAM 17 has been linked to a number of diverse signaling pathways. It cleaves ectodomains of various transmembrane proteins and regulates cytokine shedding. It also plays a role in inflammatory skin and bowel disease.

DESCRIPTION: The *ADAM17 Fluorescent Assay Kit* is provided in a convenient 96-well format, with purified ADAM17, ADAM Fluorogenic Substrate, and ADAM assay buffer for 96 enzyme reactions. The key to the *ADAM17 Fluorescent Assay Kit* is the fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for ADAM17 reactions. A sample containing ADAM17 is incubated in a reaction mixture with the fluorogenic substrate and fluorescence ($\lambda_{ex}=358\pm 10$ nm, $\lambda_{em}=455\pm 10$ nm) is measured using a plate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	ADAM17	3 μ g	-80°C	Avoid freeze/thaw cycles!
	ADAM Fluorogenic Substrate (1 mM)	50 μ l	-80°C	
78001	1X ADAM Assay Buffer	5 ml	-20°C	
79685	96-well black plate	1	Room temp.	

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:

- Scheller, Jürgen, *et al.* 2011. "ADAM17: a molecular switch to control inflammation and tissue regeneration." *Trends in Immunology* **32(8)**: 380-387.
- Blaydon, Diana C., *et al.* 2011. "Inflammatory skin and bowel disease linked to ADAM17 deletion." *New England Journal of Medicine* **365(16)**: 1502-1508.

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

- 1) Prepare the master mixture: N wells x (24.5 μ l **1x ADAM Assay Buffer 1** + 0.5 μ l **ADAM Fluorogenic Substrate** (1 mM)).
- 2) Add 25 μ l of master mixture to each well designated for the "Positive Control," "Test Inhibitor," and "Blank."
- 3) Thaw **ADAM17** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **ADAM17** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: ADAM17 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 4) Dilute **ADAM17** in **1X ADAM Assay Buffer** at 1.25 ng/ μ l (25 ng/reaction). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 5) Prepare the test inhibitor solution.

The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in **1X ADAM Assay Buffer** (at this step the compound concentration is 10-fold higher than the final concentration in 10% DMSO). To determine an IC₅₀ or to test lower concentrations of the compound, prepare a series of further dilutions in **1X ADAM Assay Buffer** containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in **1X ADAM Assay Buffer**.

- 6) Add 5 μ l of test Inhibitor solution to each well labeled as "Test Inhibitor." For the "Positive Control" and "Blank," add 5 μ l of inhibitor buffer (same solution without inhibitor compound; usually 10% DMSO in **1X ADAM Assay Buffer**).
- 7) Add 20 μ l of **1X ADAM Assay Buffer** to the wells designated "Blank."

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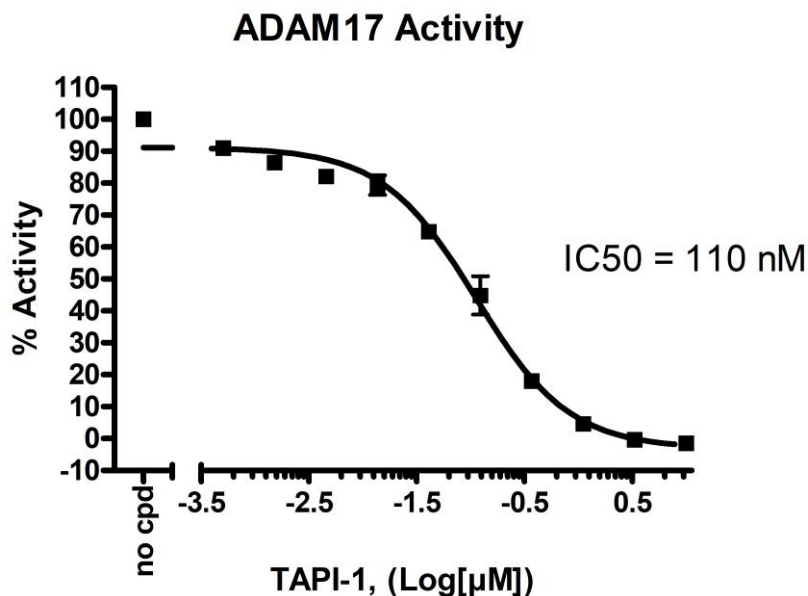
- 8) Initiate reaction by adding 20 μ l of diluted **ADAM17** (1.25 ng/ μ l) to the wells designated "Positive Control" and "Test Inhibitor." Incubate for 60 minutes at room temperature.

	Positive Control	Test Inhibitor	Blank
Master Mixture	25 μ l	25 μ l	25 μ l
Test Inhibitor	-	5 μ l	-
Inhibitor buffer	5 μ l	-	5 μ l
1x ADAM Assay Buffer	-	-	20 μ l
ADAM17 (1.25 ng/ μ l)	20 μ l	20 μ l	-
Total	50 μl	50 μl	50 μl

Step 2:

- 1) Read fluorescence at $\lambda_{ex}=358$ nm and $\lambda_{em}=455$ nm. "Blank" value is subtracted from all measurements.

Example of Assay Results:



ADAM17 enzyme activity, measured using the *ADAM17 Assay Kit*, BPS Bioscience #78000. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Fluorescent signal of positive control reaction is weak	ADAM17 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Incorrect wavelength	Use correct filters for fluorescent plate reader. Reading at wavelengths outside of 455 ± 10 nm will give a decreased signal.
Fluorescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of ADAM17 enzyme to create a standard curve.

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