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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 (λ_{ex}=358±10 nm, λ_{em}=455±10 nm) is measured using a plate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	ADAM17	3 µg	-80°C	***Avoid freeze/ thaw cycles!***
	ADAM Fluorogenic Substrate (1 mM)	50 µl	-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:
1. Scheller, Jürgen, *et al.* 2011. "ADAM17: a molecular switch to control inflammation and tissue regeneration." *Trends in Immunology* **32(8):** 380-387.
2. 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 µl **1x ADAM Assay Buffer 1** + 0.5 µl **ADAM Flurogenic Substrate** (1 mM)).

2) Add 25 µl 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/µl (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 µl of test Inhibitor solution to each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of inhibitor buffer (same solution without inhibitor compound; usually 10% DMSO in **1X ADAM Assay Buffer**).

7) Add 20 µl of **1X ADAM Assay Buffer** to the wells designated “Blank."

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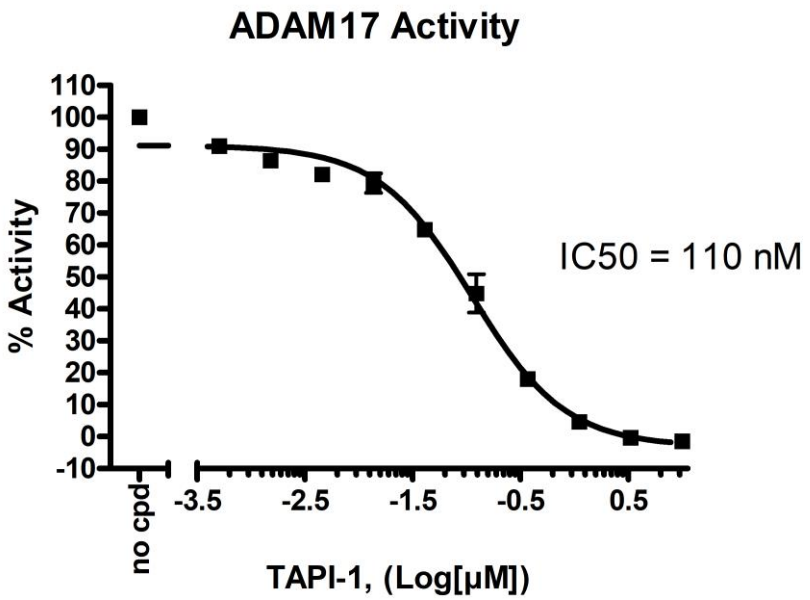
8) Initiate reaction by adding 20 µl of diluted **ADAM17** (1.25 ng/µl) to the wells designated “Positive Control” and "Test Inhibitor.” Incubate for 60 minutes at room temperature.

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

Step 2:

1) Read fluorescence at λ_{ex}=358 nm and λ_{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.