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Data Sheet

ADAM10 Fluorogenic Assay Kit

Catalog #78007
Size: 96 reactions

BACKGROUND: ADAM10 or ADAM Metallopeptidase Domain 10, is an enzyme that is involved in a diverse array of signaling pathways. It is part of the ADAM family of disintegrins and metalloproteases. It also plays a role in inflammatory disease and cancer.

DESCRIPTION: The *ADAM10 Fluorogenic Assay Kit* is provided in a convenient 96-well format, with purified ADAM10, ADAM Fluorogenic Substrate, and ADAM assay buffer for 96 enzyme reactions. The key to the *ADAM10 Fluorogenic Assay Kit* is the fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for ADAM10 reactions. A sample containing ADAM10 is incubated in a reaction mixture with the fluorogenic substrate and fluorescence (λ_{ex}=485±10 nm, λ_{em}=530±10 nm) is measured using a plate reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	ADAM10*	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.	

**The concentration of ADAM10 is lot-specific and will be indicated on the tube containing the enzyme.*

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.

REFERENCE(S):
1. Scheller, J., *et al.* 2011. "ADAM10: a molecular switch to control inflammation and tissue regeneration." *Trends in Immunology* **32(8):** 380-387.
2. Blaydon, D.C., *et al.* 2011. "Inflammatory skin and bowel disease linked to ADAM10 deletion." *New England Journal of Medicine* **365(16):** 1502-1508.

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

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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 2** (1 mM)).

2) Add 25 µl of master mixture to each well designated for the "Positive Control," "Test Inhibitor," and "Blank."

3) Thaw **ADAM10** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **ADAM10** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: ADAM10 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **ADAM10** 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.

	Positive Control	Test Inhibitor	Blank
Master Mixture	25 µl	25 µl	25 µl
Test Inhibitor	–	5 µl	–
10% DMSO in water (Inhibitor buffer)	5 µl	–	5 µl
1x ADAM Assay Buffer	-	-	20 µl
ADAM10 (1.25 ng/µl)	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**

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6) Add 5 µl of test inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of 10% DMSO in water (inhibitor buffer).

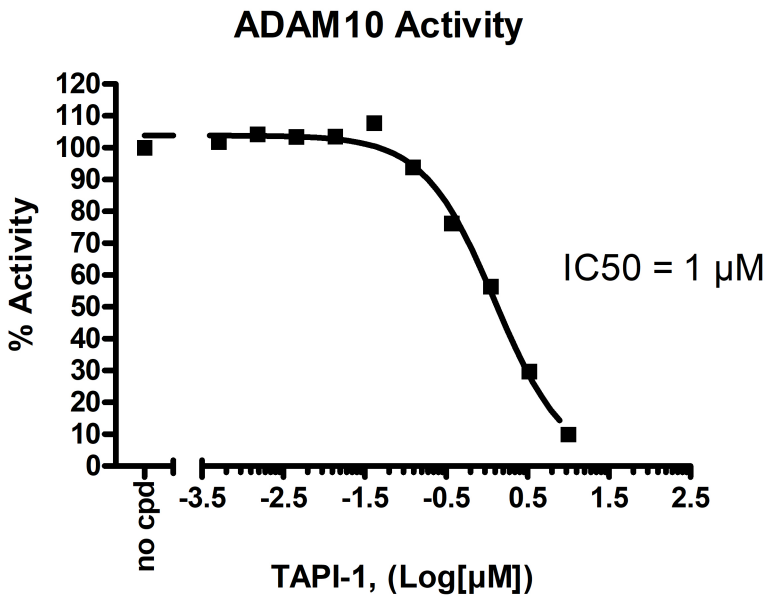
7) Add 20 µl of **1X ADAM Assay Buffer** to the wells designated “Blank."

8) Initiate reaction by adding 20 µl of diluted **ADAM10** (1.25 ng/µl) to the wells designated “Positive Control” and "Test Inhibitor.” Incubate for 60 minutes at room temperature.

Step 2:

1) Read fluorescence at λ_{ex}=485 nm and λ_{em}=530 nm. “Blank” value is subtracted from all measurements.

Example of Assay Results:



Inhibition of ADAM10 by TAPI-1, measured using the ADAM10 assay kit (BPS Bioscience #78007). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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Related Products:

Product	Cat. #	Size
ADAM17 Fluorogenic Assay Kit	78000	96 rxns.
Fluorogenic MMP3 (K45E) Assay Kit	79907	384 rxns.
Fluorogenic MMP1 Assay Kit	79983	96 rxns.
Fluorogenic MMP2 Assay Kit	79918	96 rxns.
Fluorogenic MMP8 Assay Kit	79929	96 rxns.
Fluorogenic MMP9 (Q279R) Assay Kit	79915	96 rxns.
Fluorogenic MMP10 Assay Kit	79986	96 rxns.
Fluorogenic MMP13 Assay Kit	79991	96 rxns.
MMP1, His-Tag (Human)	80214	20 µg
MMP2, His-Tag (Human)	80213	20 µg
MMP3(K45E), His-Tag (Human)	11346	100 µg
MMP8, His-Tag (Human)	100552	100 µg
MMP9(Q279R), His-Tag (Human)	80215	20 µg