Data Sheet

Fluorogenic MALT1 Assay Kit

Catalog #79809

Size: 96 reactions

BACKGROUND: MALT1 (Mucosa-associated lymphoid tissue lymphoma translocation protein 1) is a cysteine protease that structurally resembles caspases. MALT1 is a member of the CARMA1-BCL10-MALT1 (CBM) complex that mediates NF-κB activation downstream of the B cell receptor, T cell receptor and ITAM-coupled natural killer cell receptors. MALT1 cleavage activity is essential to T cell and B cell activation and is linked to the pathogenesis of activated B cell-like diffuse large B cell lymphoma (ABC-DLBCL), a chemoresistant form of DLBCL.

DESCRIPTION: The *Fluorogenic MALT1 Assay Kit* is designed to measure MALT1 activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The MALT1 assay kit comes in a convenient 96-well format, with purified MALT1 enzyme, fluorogenic substrate, and MALT1 assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100360	Recombinant Human MALT1	30 µg	-80°C	***Avoid freeze/ thaw cycles!***
79810	1 mM MALT1 Substrate	100 µl	-80°C	
79811	1X MALT1 Assay Buffer	10 ml	-20°C	
	0.5 M DTT	200 µl	-20°C	
79685	Black, low binding black microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading exc/em=360nm/460nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

REFERENCE(S):

Fontan, L. *et al., Cancer Cell*. 2012 Dec 11; **22(6):** 812–824.
Nagel, D. *et al., Cancer Cell*. 2012 Dec 11;**22(6):**825-837.
Rebeaud, F. *et al., Nat Immunol*. 2008; **9:**272-281

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Add 0.5 M DTT to 1X assay buffer so final DTT concentration is 1 mM. For example, add 10 µl of 0.5M DTT to 5 ml 1X assay buffer. (DTT should be added freshly. Prepare only enough DTT-containing buffer as required for the assay. Store the remaining 1X assay buffer at -20°C).

2) Thaw MALT1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MALT1 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: MALT1 enzyme is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

3) Dilute MALT1 in 1x assay buffer (with 1 mM DTT) at 15 ng/µl (300 ng per reaction).

4) Add 20 µl diluted MALT1 enzyme solution to wells designated as “Positive Control” and “Test Sample”. Add 20 µl 1X assay buffer to the “Blank” wells.

5) Prepare the 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 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 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 assay buffer.

6) Add 5 µl inhibitor solution to each well designated “Test Sample”. Add 5 µl 1X assay buffer or 10% DMSO (depending on which inhibitor solution is used) to “Blank” and “Positive Control” wells.

7) Incubate the enzyme with the compound solution at room temperature for 30 minutes.

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Component	Positive Control	Test Sample	Blank
MALT1 (15 ng/µl)	20 µl	20 µl	–
1X Assay Buffer	–	–	20 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)*	5 µl	–	5 µl
Incubate at room temperature for 30 minutes			
Substrate solution	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

8) Prepare the substrate solution: N wells × (24 µl 1X assay buffer (with DTT) + 1 µl 1 mM MALT1 Substrate).

9) Add 25 µl of the substrate solution to each well (Final concentration of the MALT1 substrate in a 50 µl reaction is 20 µM).

10) Incubate at room temperature for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at a wavelength 360 nm and detection of emission at a wavelength 460 nm. The fluorescence intensity can also be measured kinetically. “Blank” value is subtracted from all other values.

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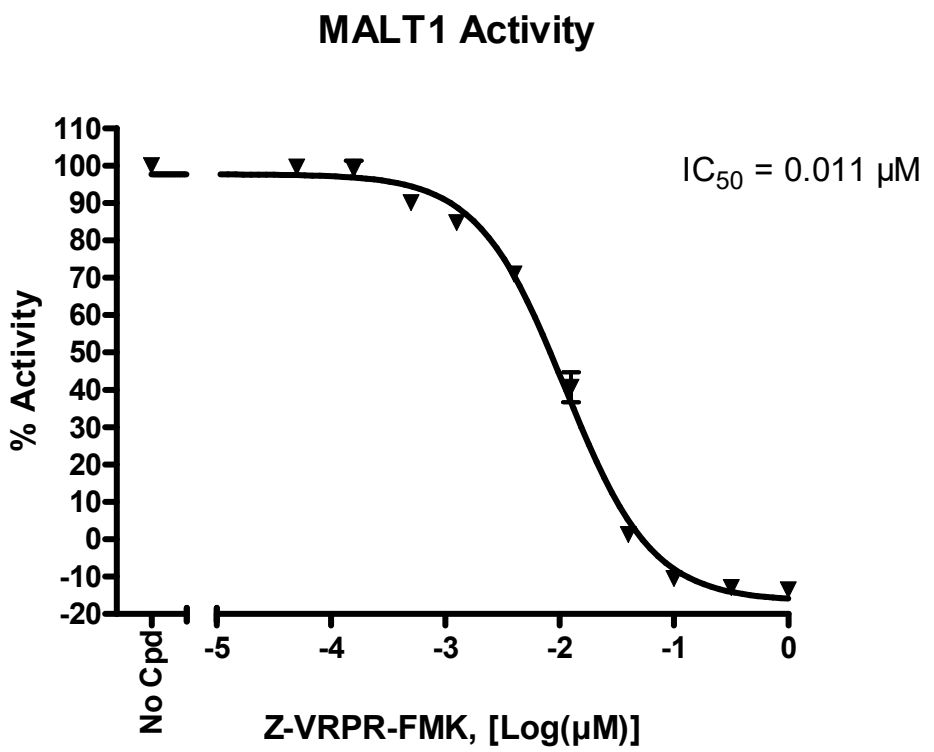
EXAMPLE OF ASSAY RESULTS:



MALT1 enzyme activity, measured using the *Fluorogenic MALT1 Assay Kit (#79809).* Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact info@amsbio.com.*

RELATED PRODUCTS

Product	Cat. #	Size
MALT1, FLAG-Tag (Human)	100360	20 µg
MALT1, FLAG-Tag (Mouse)	100054	20 µg
NF-κB reporter (Luc) – NIH/3T3 Cell line	79469	1 vials
A20, His-tag	80393	100 µg

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