ab155889

TACE Inhibitor Screening Kit

Instructions for Use

For the sensitive and accurate measurement of TACE inhibitors in various samples

This product is for research use only and is not intended for diagnostic use.

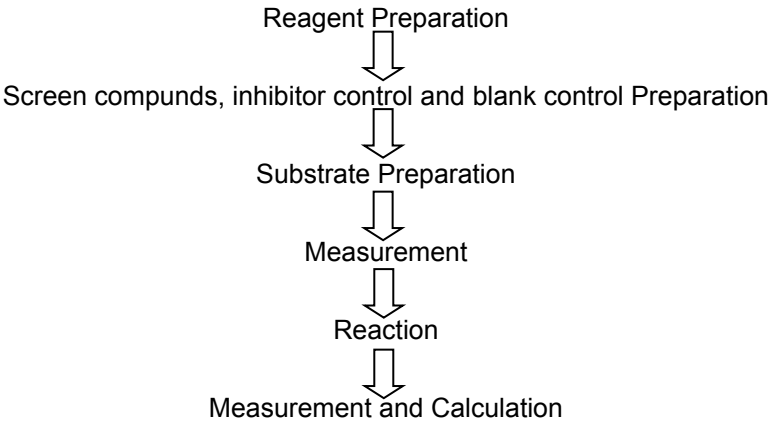
Table of Contents

1. Overview

The TACE (tumor necrosis factor-α-converting enzyme), also called ADAM metallopeptidase domain 17 (ADAM17), is a 70-kDa enzyme that belongs to the ADAM protein family of disintegrins and metalloproteases. TACE is believed to be involved in the processing of tumor necrosis factor alpha (TNF-α) at the surface of the cell, and from within the intracellular membranes of the trans-Golgi network. This process, which is also known as 'shedding', involves the cleavage and release of a soluble ectodomain from membrane-bound pro-proteins (such as pro-TNF-α), and is of known physiological importance.

In Abcam's TACE inhibitor screening Kit, TACE hydrolyzes the specific FRET substrate to release the quenched fluorescent group, which can be detected fluorometrically at Ex/Em = 318/449 nm. In the presence of the potent TACE inhibitor, the hydrolyzation of substrate will be impeded. The kit provides a rapid, simple, sensitive and reliable test suitable for high-throughput screening of TACE inhibitors and can be modified to check the relative TACE activity. Inhibitor Control GM6001 is included to compare the efficacy of test inhibitors.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer	25 mL
Substrate	0.2 mL
TACE enzyme (20 µg)	1 vial
Inhibitor Control (0.1 mM GM6001)	20 µL

Store the kit at -20°C and protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

Please read the entire protocol before performing the assay.

B. Additional Materials Required

- 96-well plate with flat clear bottom, black wall plates are preferred for fluorescence reading
- Fluorescence plate reader
- Multi-channel pipette

4. Assay Protocol

A. Reagent Preparation

1. TACE Enzyme:

Dissolve the TACE enzyme with 220 µl Assay Buffer. Aliquot and store the stock solution at -80°C (preferable) or -20°C. Avoid repeated freeze/thaw cycles. Use within one week.

B. TACE Inhibitor Screening Assay Protocol

1. Enzyme preparation:

For each well, prepare 50 µl TACE enzyme solution.

48 µl Assay Buffer

2 µl TACE enzyme

2. Screen compounds, inhibitor control and blank control preparations:

Dissolve candidate inhibitors into proper solvent. Dilute to 4X the desired test concentration with Assay Buffer. For GM6001 Inhibitor Control, dilute GM6001 1:24 with Assay buffer. Add 25 µl diluted test inhibitors, Inhibitor Control or Assay Buffer into TACE enzyme wells as sample screen [S], Inhibitor Control (GM6001), or Enzyme Control [EC] (no inhibitor). Mix well, and incubate for 5 minutes at 37°C.

3. Substrate preparation:

For each well, prepare a total 25 µl substrate solution

23 µl Assay Buffer

2 µl Substrate

Mix, add 25 µl substrate solution into each well. Mix well.

4. Measurement:

Read fluorescence (R_1) at Ex/Em = 318/449 nm. Incubate the reaction at 37°C for 30 min, protected from light and measure again fluorescence (R_2) at Ex/Em = 318/449 nm.

5. Data Analysis

Calculation: The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta RFU = R_2 - R_1$. Set the ΔRFU of Enzyme Control [EC] as 100%, and calculate the relative % inhibition of the test inhibitors as:

$$\% \text{ Inhibition} = \frac{\Delta RFU \text{ of EC} - \Delta RFU \text{ of S}}{\Delta RFU \text{ of EC}} \times 100\%$$

Note: It is recommended to read kinetically to choose the R_1 and R_2 at linear range.

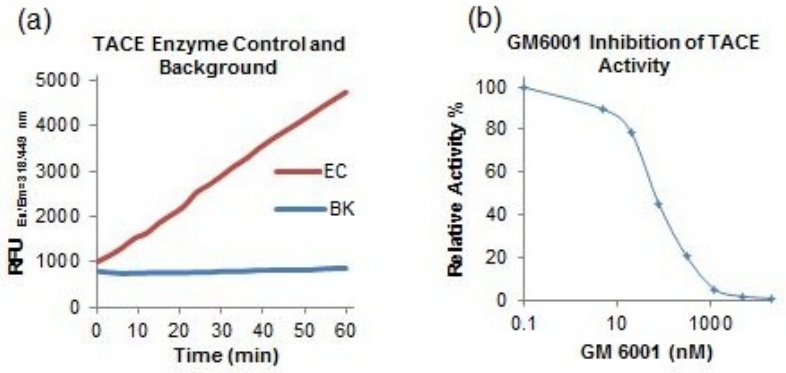


Figure (a) TACE Enzyme Control and Background **(b)** GM6001 Inhibition of TACE Activity.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp