TACE Inhibitor Screening Assay Kit (Fluorometric)

(Catalog #BN00629; 100 assays; Store kit at -20°C)

I. Introduction: 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 Assay Genie'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.

II. Kit Contents:

Components	BN00629	Cap Code	Part Number
TACE Assay Buffer	25 ml	WM	BN00629-1
TACE Substrate	0.2 ml	Red	BN00629-2
TACE Enzyme (20 µg)	1 vial	Green	BN00629-3
Inhibitor Control (0.1 mM GM6001)	20 µl	Purple	BN00629-4

III. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Preparation:

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.

V. 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

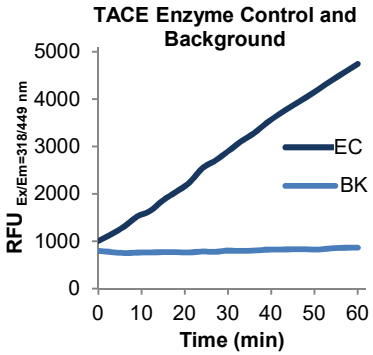
   Mix, add 25 µl substrate solution into each well. Mix well.

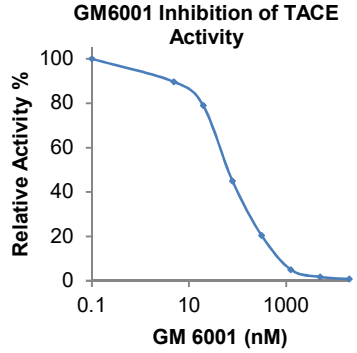
4. **Measurement:** Read fluorescence (R1) at Ex/Em = 318/449 nm. Incubate the reaction at 37°C for 30 min, protected from light and measure again fluorescence (R_2) at Ex/Em = 318/449 nm.

5. **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:

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

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





FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		