

ab284516 – TEV Protease Inhibitor Screening Kit (Fluorometric)

For the screening of potential TEV Protease inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab284516>

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
TEV Protease Assay Buffer	25 mL	-20°C
TEV Protease	100 µL	-20°C
TEV Protease Substrate	100 µL	-20°C
TEV Protease Inhibitor (2 mM)	20 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- White 96-well plate with flat bottom.
- Multi-well spectrofluorometer

Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.

TEV Protease Assay Buffer: Bring to room temperature before use. Store at 4°C or -20°C.

TEV Protease: Aliquot and store at -20°C. Avoid repeated freeze/thaw.

Assay Protocol

TEV Protease Enzyme Solution Preparation:

1. For each well, prepare 50 µL of TEV Protease enzyme solution.

	Enzyme Solution
TEV Protease Assay Buffer	49 µL
TEV Protease enzyme	1 µL

2. Mix well and add 50 µL/well into desired wells in a 96-well microtiter plate

Screening Compounds, Inhibitor Control & Blank Control Preparations:

1. Dissolve test inhibitors into proper solvent.
2. Dilute to 10X the desired test concentration with TEV Protease Assay Buffer. Add 10 µL diluted test inhibitors (Sample, S) or TEV Protease Assay Buffer (Enzyme Control, EC) into TEV Protease enzyme containing wells.
3. For Inhibitor Control (IC), add 1 µL TEV Protease Inhibitor and 9 µL TEV Protease Assay Buffer into TEV Protease enzyme well(s). Incubate at room temperature for 15 min.

Δ Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on enzymatic activity is a concern, prepare a solvent control well with the same final

concentration of the solvent as in the inhibitor sample, as solvent control (SC).

Substrate Mix:

1. For each well, prepare 40 µL of the substrate solution:

	Substrate Mix
TEV Protease Assay Buffer	39 µL
TEV Protease Substrate	1 µL

2. Mix & add 40 µL of TEV Protease Substrate solution into Enzyme Control, Inhibitor Control, solvent control & sample wells. Mix well.

Measurement

Measure fluorescence (Ex/Em = 490/560 nm) in a kinetic mode for 1 hour at 34°C. The assay can be run at any temperature 22-37°C, but the sensitivity will vary accordingly. Choose two time points (T₁ & T₂) where the corresponding RFUs (RFU₁ and RFU₂) are in a linear range. Calculate ΔRFU and ΔT.

Calculation:

Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU₂-RFU₁) values with the time ΔT (T₂-T₁).

$$\% \text{ Relative Inhibition} = \frac{\text{slope of EC} - \text{slope of S}}{\text{slope of EC}} \times 100$$

Δ Notes:

- a) Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S).
- b) If Solvent control (SC) values are significantly different from the EC, use these values in the equation above instead of EC.
- c) Irreversible inhibitors that inhibit the TEV Protease activity completely at the tested concentration will have ΔRFU = 0 and thus the % Relative Inhibition will be 100%.

Technical Support

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