

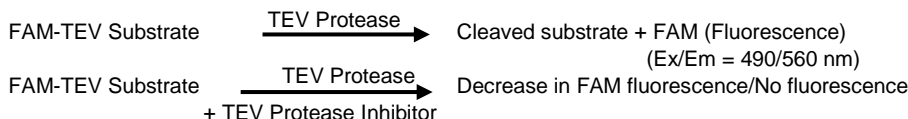
TEV Protease Inhibitor Screening Kit (Fluorometric)

(Catalog # K843-100; 100 assays, Store kit at -20°C)

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I. Introduction:

TEV Protease (EC: 3.4.22.44) is a cysteine protease that recognizes the cleavage site of Glu-Xaa- Xaa-Y- Xaa-Gln-(Gly/Ser) and cleaves between Gln and Gly/Ser. The optimal sequence is Glu-Asn-Leu-Tyr-Phe-Gln-Ser/Glycine (ENLYFQS/G). TEV Protease has high specificity and great stability and is active over a wide range of temperatures (4-37°C) with an optimal activity at 34°C. BioVision's Inhibitor Screening Kit utilizes the ability of TEV Protease to cleave a synthetic Fluorescein-based peptide substrate to release fluorescein which can be easily quantified using a fluorometer or fluorescence microplate reader (Ex/Em = 490/560 nm). In the presence of a TEV Protease-specific inhibitor, the cleavage of the substrate is reduced/abolished resulting in decrease or total loss of the fluorescence. This simple and high-throughput adaptable assay kit can be used to screen/study/characterize potential inhibitors of TEV Protease.



II. Applications:

- Screening/studying/characterizing inhibitors of TEV Protease.

III. Kit Contents:

Components	K843-100	Cap Code	Part Number
TEV Protease Assay Buffer	25 ml	WM	K843-100-1
TEV Protease	100 µl	Green	K843-100-2
TEV Protease Substrate	100 µl	Brown	K843-100-3
TEV Protease Inhibitor (2 mM)	20 µl	Purple	K843-100-4

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **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.

VI. TEV Protease Inhibitor Screening Protocol:

1. **TEV Protease Enzyme Solution Preparation:** For each well, prepare 50 µl of TEV Protease enzyme solution.

49 µl TEV Protease Assay Buffer
1 µl TEV Protease enzyme

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

2. **Screening Compounds, Inhibitor Control & Blank Control Preparations:** Dissolve test inhibitors into proper solvent. 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. 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.

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).

3. **TEV Protease Substrate Preparation:** For each well, prepare 40 µl of the substrate solution.

39 µl TEV Protease Assay Buffer
1 µl TEV Protease Substrate

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

4. **Measurement:** Measure fluorescence (Ex/Em = 490/560 nm) in a kinetic mode for 1 hr 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.
5. **Calculations:** 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).

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

Note:

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

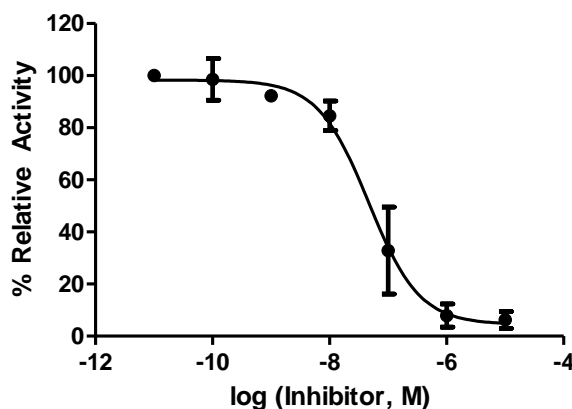


Figure: Inhibition of TEV Protease activity by TEV Protease Inhibitor ($\text{IC}_{50} = 46 \text{ nM}$). Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

TEV Protease Activity Fluorometric Assay Kit (K842)	EZCut™ TEV Protease, Recombinant (7847)
HIV Protease Inhibitor Screening Kit (K826)	Active HIV1 Protease Recombinant (GST-tagged) (7849)
Cathepsin L Activity Fluorometric Assay Kit (K142)	Cathepsin L Antibody (3192)
Cathepsin L (Cleaved) Antibody (3741)	Cathepsin L (Cleaved) Blocking Peptide (3741BP)
Cathepsin L Blocking Peptide (3192BP)	Cathepsin L, human recombinant (1135)
Cathepsin B (1021)	Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin B Antibody (3190)	Cathepsin B Inhibitor Screening Kit (K147)
Cathepsin D (1022)	Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin D Antibody (3191R)	Cathepsin D Blocking Peptide (3191RBP)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)	Cathepsin F Antibody (3371)
Cathepsin F Blocking Peptide (3371BP)	Cathepsin G Activity Assay Kit, Fluorometric (K146)
Cathepsin G Antibody (3370)	Cathepsin G Inhibitor (1982)
Cathepsin G Substrate (2206)	Cathepsin G, human neutrophil (4713)
Cathepsin G Activity Fluorometric Assay Kit (K146)	Cathepsin H (1023)
Cathepsin H Activity Fluorometric Assay Kit (K145)	Cathepsin K Activity Fluorometric Assay Kit (K141)
Procathepsin K, human recombinant (1026)	Procathepsin K, mouse recombinant (1027)
Procathepsin K, rat recombinant (1029)	Cathepsin K Antibody (3588, 3368)
Cathepsin K Blocking Peptide (3588BP, 3368BP)	Human CellExp™ Cathepsin S, human recombinant (7277)
Cathepsin S Activity Fluorometric Assay Kit (K144)	Cathepsin S Inhibitor Screening Kit (K149)
Cathepsin S Antibody (3366, 3366R)	Cathepsin S Blocking Peptide (3366R)

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