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TEV Protease Activity Assay Kit (Fluorometric)

9/17

(Catalog # K842-100; 100 assays, Store kit at -20°C)

I. Introduction:

TEV Protease (EC: **3.4.22.44,** Tobacco Etch Virus protease) 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 TEV Protease Activity assay 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). This assay kit is simple, rapid and can detect TEV Protease activity as low as 50 ng/well in viral lysates and purified protein samples.

FAM-TEV Substrate $\xrightarrow{\text{TEV Protease}}$ Cleaved substrate + FAM (Fluorescence) (Ex/Em = 490/560 nm)

II. Applications:

- Detection of TEV Protease activity in viral lysates
- Determine activity of purified TEV Protease

III. Kit Contents:

Components	K842-100	Cap Code	Part Number
TEV Protease Assay Buffer	25 ml	WM	K842-100-1
TEV Protease Dilution Buffer	10 ml	NM	K842-100-2
1 M DTT	100 µl	Blue	K842-100-3
TEV Protease (Positive Control)	10 µl	Green	K842-100-4
TEV Protease Substrate	100 µl	Brown	K842-100-5
5-FAM Standard (100 µM in DMSO)	100 µl	Yellow	K842-100-6

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectroflurometer.
- **Optional:** BCA Protein Assay Kit - Reducing Agent Compatible **(Cat. # K818-1000, K819-250 or equivalent).**

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 entire protocol before performing the assay.

- **TEV Protease Assay Buffer:** Add 100 µl of 1 M DTT to the TEV Protease Assay Buffer. Mix well. Store at -20°C. Bring to room temperature before use.
- **TEV Protease (Positive Control):** Aliquot and store at -80°C. Avoid repeated freeze/thaw.

VI. TEV Protease Activity Assay Protocol:

1. **Sample Preparation:** Prepare TEV Protease viral lysates by appropriate methods, e.g. collect the virus-infected cells by spinning them for 10 min. at 1000 x g at 4°C. Resuspend the pellet in desired medium. Lyse the cells by freezing (-70°C, use dry ice/methanol) and thawing (37°C water bath) for about 5 cycles. After each cycle, vortex for 30 sec. Spin the cell debris at (1000 x g) for 10 min. and collect the supernatant as viral lysate. Use upto 50 µl lysates immediately to measure TEV Protease activity. If necessary, dilute the lysate with TEV Protease Dilution Buffer. Do a pilot experiment with different volumes/concentrations of the sample, to select the optimal amount, which gives a final reading in the linear range of the standard curve. For a positive control, dilute 5 µl of TEV Protease with 195 µl of TEV Protease Dilution Buffer to obtain a solution of 25 ng/µl. Use 2-10 µl per well. Bring the volume of all wells to 50 µl with the TEV Protease Assay Buffer. The diluted TEV Protease solution can be stored at 4°C for up to 2 days with minor loss in the protease activity. For long term storage, it must be stored at -80°C.

   Notes:

   a. The sample lysate must be used immediately to measure TEV Protease activity. If not, the lysate must be snap frozen and stored at -80°C containing 20% glycerol.

   b. Optional: Measure the amount of protein in the lysate using BCA Protein Assay Kit - Reducing Agent Compatible (Cat. K818-1000, K819-250 or equivalent).

   c. Optional: For samples with potentially high backgrounds, prepare parallel sample well(s) as sample background control. Use the same amount of the lysate or purified enzyme as in the sample well. Adjust the final volume to 100 µl with TEV Protease Assay Buffer.

2. **Standard Curve Preparation:** To obtain 10 µM of 5-FAM Standard dilute 10 µl of 100 µM 5-FAM Standard with 90 µl TEV Protease Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of diluted 10 µM 5-FAM Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with TEV Protease Assay Buffer to generate 0, 20, 40, 60, 80, and 100 pmol/well of 5-FAM Standard respectively. Mix well.

   Optional: If the target sample has low protease activity, prepare a standard curve of 2-10 pmol/well. For that, prepare 1 µM standard solution by diluting 10 µl of 100 µM standard with 990 µl of TEV Protease Assay Buffer. Then, add 2, 4, 6, 8, and 10 µl of 1 µM

standard solution in to a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with TEV Protease Assay Buffer to generate 0, 2, 4, 6, 8, and 10 pmol/well of 5-FAM Standard respectively.

3. **Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix as below:

   49 µl TEV Protease Assay Buffer
   1 µl TEV Protease Substrate

   Mix & add 50 µl of TEV Protease Substrate solution into each Sample, and Positive Control well. Mix well.

   Note: Don't add substrate mix to the sample Background Control and Standard wells.

4. **Measurement:** Immediately, start measuring the fluorescence (Ex/Em = 490/560 nm) in a kinetic mode for upto 15-30 min at 34°C. The assay can be run at temperatures between 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**: Measure the fluorescence of the standards in an end point mode. Subtract 0 Standard reading from all readings. Plot the 5-FAM Standard Curve. Apply sample's ΔRFU to 5-FAM Standard Curve to obtain corresponding TEV Protease amount (**B**, in pmol) and calculate the activity of TEV Protease in the sample as:

$$\textbf{Sample TEV Protease Activity} = \frac{B}{\Delta T \times V} \times \textbf{Dilution Factor} = \frac{\textbf{pmol}}{\textbf{min}}/\textbf{ml}$$

Optional:

$$\textbf{TEV Protease Activity Per mg of Protein} = \frac{B}{\Delta T \times M} \times \textbf{Dilution Factor} = \frac{\textbf{pmol}}{\textbf{min}}/\textbf{mg}$$

Where: **B** = TEV Protease amount from the 5-FAM Standard Curve (pmol)
V = sample volume initially added into the reaction well (ml)
M = Amount of protein in the sample (mg)
Δ**T** = reaction time (min)

Unit Definition: One unit of TEV protease is the amount of enzyme that produces 1 µmol of 5-FAM metabolite per minute at pH 8.0 at 34 °C.

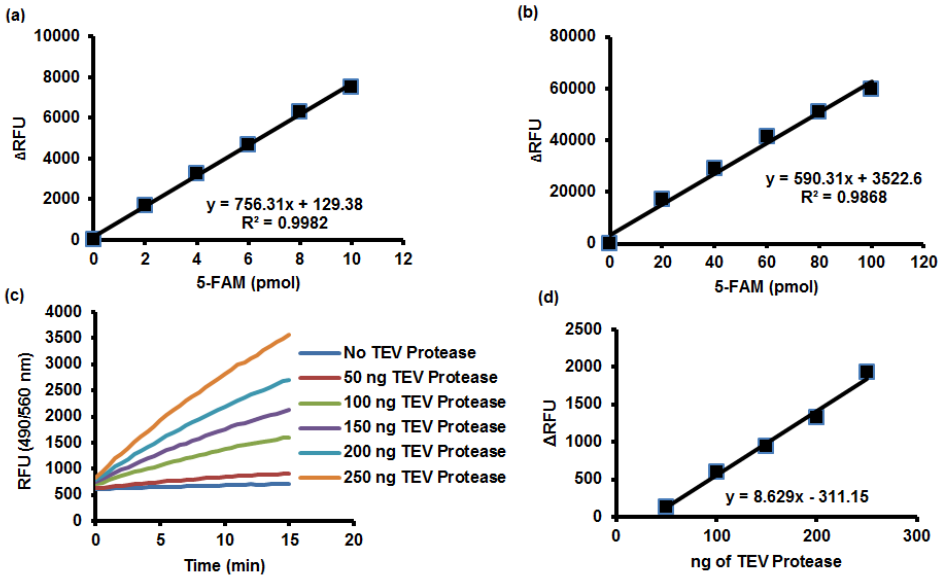

Figure: 5-FAM Standard Plots (2-10 pmol, a, and 20-100 pmol, b), kinetics progress curves for TEV Protease as a positive control (c) and calculated ΔRFU (after 10 min) for different amounts of TEV Protease (d) are presented. Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

TEV Protease Inhibitor Screening Kit (K843)
HIV Protease Inhibitor Screening Kit (K826)
Cathepsin L Activity Fluorometric Assay Kit (K142)
Cathepsin B Inhibitor Screening Kit (K147)
Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)
Cathepsin G Activity Fluorometric Assay Kit (K146)
Cathepsin K Activity Fluorometric Assay Kit (K141)
EZCut™ TEV Protease, Recombinant (7847)
Active HIV1 Protease Recombinant (GST-tagged) (7849)
Cathepsin B Activity Fluorometric Assay Kit (K140)
Cathepsin D Activity Fluorometric Assay Kit (K143)
Cathepsin G Activity Assay Kit, Fluorometric (K146)
Cathepsin H Activity Fluorometric Assay Kit (K145)
Cathepsin S Activity Fluorometric Assay Kit (K144)

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