Aurora Biolabs
High Purity Protein for Research

TEV Protease Activity Assay Kit

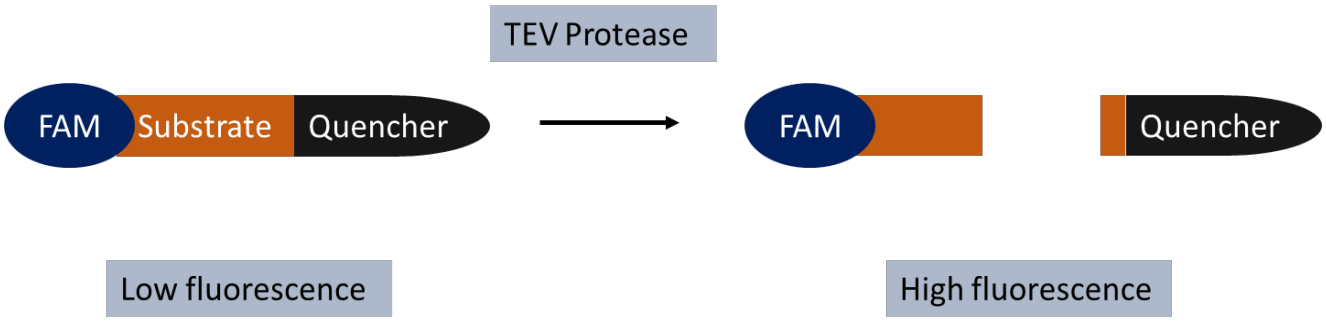
Catalog Number: 190001AK

Background

Tobacco Etch Virus protease (TEV protease) is a highly sequence specific cysteine protease. It has a strict 7 amino acid cleavage recognition sequence of Glu-Asn-Leu-Tyr-Phe-Gln ↓ (Gly/Ser). The high specificity makes this protease excellent for the removal of affinity-tags from purified recombinant proteins.

Assay Principle

The TEV Protease Activity Assay kit is a fluorogenic-based assay to measure TEV protease activity. The kit contains a TEV protease substrate that is labeled with fluorophore FAM and a quencher. Proteolytic activity of TEV protease cleaves the substrate and releases the FAM, resulting in the production of bright fluorescence which can be measured using a fluorescence reader at ex/em of 490 nM/520 nm. TEV protease activity then can be calculated in accordance with the fluorescence intensity. Purified TEV protease is included in the kit as a positive control.



Application

Measure TEV protease activity.

Plate Reader

A microplate reader capable of measuring fluorescence intensity is required.

Components

Catalog number	Item	Amount	Storage
190001B	Assay buffer	25 mL	-20°C
190001	Purified TEV protease	4 µg X 2 vials	-80°C
190001F	Fluorogenic substrate	200 µL	-80°C
190001S	5-FAM standard (1 mM)	10 µL	-80°C
	96-well microplate, black	1	Room temperature

Materials needed but not supplied

1. Microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

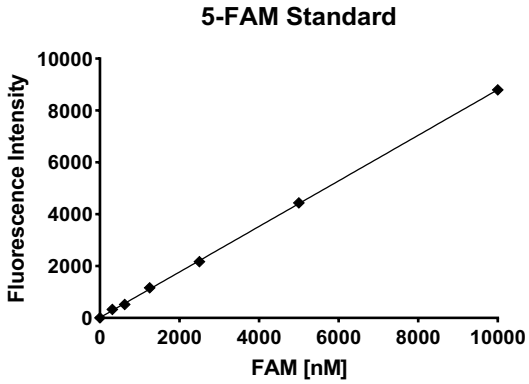
Assay protocol

A. Prepare assay buffer containing 1 mM DTT

For example, mix 998 µl of assay Buffer and 2 µl of 0.5 M DTT. Make only enough DTT-containing assay buffer as needed for the assay. Store the remaining assay buffer at -20°C.

B. Making 5-FAM standard curve

1. Dilute 1 mM 5-FAM to 20 µM with the assay buffer prepared at step A (assay buffer A).
2. Make 2-fold series of dilutions with the assay buffer a to get 10, 5, 2.5 1.25, 0.625, 0.3125 and 0 µM solutions.
3. Aliquot 50 µL of the diluted solution to each well (96-well plate).
4. Dilute substrate solution 25-fold with the assay buffer A.
5. Add 50 µl of diluted substrate to each well.
6. Measure fluorescent intensity at excitation of 490 nm and emission of 520 nm.
7. Use the same machine settings when measure TEV protease activity afterwards.



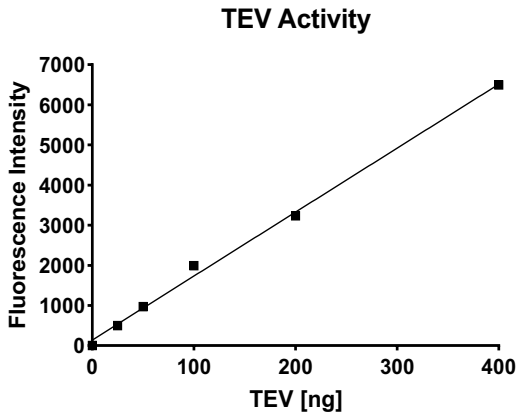
C. Measure TEV protease positive control activity

1. Thaw TEV protease protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: TEV protease protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. **Do not re-use the diluted protein.**
2. Dilute the TEV protein 125-fold with the assay buffer A (from 1000 ng/µL to 16 ng/µL). Then, make a further dilution to 8, 4, 2, 0.5, 0 ng/µL.
3. Add 50 µl of diluted protein solution to each well (Test amount of the protein will be 400, 200, 100, 50, 25 and 0 ng per reaction).

We recommend to run the reaction in duplicate.

4. Dilute substrate solution 25-fold with assay buffer A.
5. Add 50 µl of diluted substrate to each well.
6. Incubate at room temperature for 1 hour.
7. Measure fluorescent intensity at excitation of 490 nm and emission of 520 nm.
8. Plot fluorescent intensity versus protein concentration on a graph as below (subtract the average fluorescent intensity readings in the 0 ng wells from all of other wells to remove fluorescence background).



D. Measure TEV protease activity

1. Dilute TEV protease protein to 8, 4, 2, 0.5, 0 ng/µL with the assay buffer A.
2. Add 50 µl of diluted protein solution to each well (Test amount of the protein will be 400, 200, 100, 50, 25 and 0 ng per reaction).

   We recommend to run the reactions in duplicate.
3. Dilute substrate solution 25-fold with assay buffer A.
4. Add 50 µl of diluted substrate to each well.
5. Incubate at room temperature for 1 hour.
6. Measure fluorescent intensity at excitation of 490 nm and emission of 520 nm.
7. Plot fluorescent intensity versus protein concentration on a graph as below (subtract the average fluorescent intensity readings in the 0 ng wells from all of other wells to remove fluorescence background).