Version 4a, Last updated 26 March 2024

ab211109 TEV Protease Activity Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of TEV Protease activity in a variety of samples.

This product is for research use only and is not intended for diagnostic use.

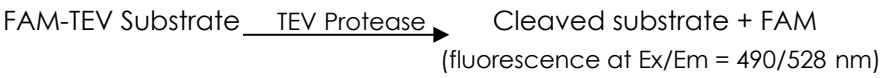
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

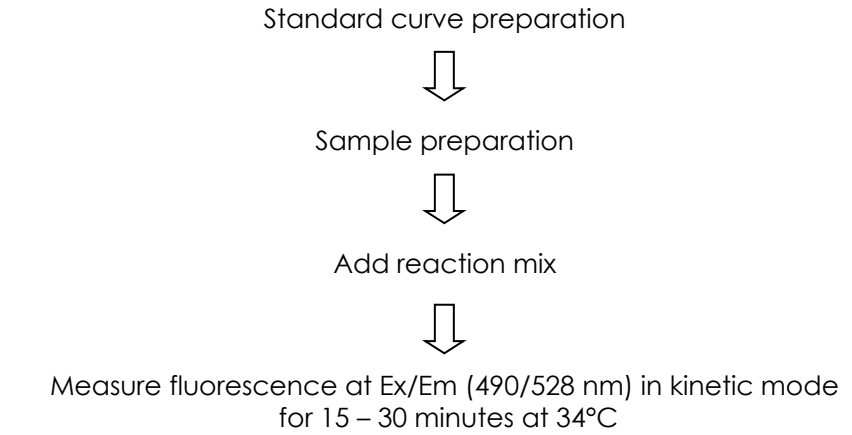
TEV Protease Activity Assay Kit (Flurometric) (ab211109) provides a convenient method for detecting TEV (Tobacco Etch Virus) Protease activity in cell lysates from infected individuals, as well as from purified proteins. The assay is based on the ability of TEV Protease to cleave a synthetic Fluorescein-based peptide substrate to release fluorescein which can be easily quantified using a fluorescence microplate reader at Ex/Em = 490/528 nm.

This assay kit is simple, rapid and can detect as low as 50 ng TEV Protease activity in samples.



TEV Protease (Tobacco Etch Virus 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.

2. Protocol Summary



**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
TEV Protease Assay Buffer	25 mL	-20°C	-20°C
TEV Protease Dilution Buffer	10 mL	-20°C	-20°C
DTT II/DTT (1M)	100 µL	-20°C	-20°C
TEV Protease Positive Control/TEV Protease (Positive Control)	10 µL	-20°C	-80°C
TEV Protease Substrate	200 µL	-20°C	-20°C
5-FAM Standard/5-FAM Standard (100 µM in DMSO)	100 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 490/528 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with black flat bottom
- Dry ice/methanol
- BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 DTT II/DTT (1 M) (100 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C. Keep on ice while in use.

9.2 TEV Protease Assay Buffer (25 mL):

Equilibrate to room temperature before use.

Prior starting the assay, add 100 µL of 1 M DTT II/DTT to the TEV Protease Assay Buffer to prepare **Assay Buffer/DTT solution**. Mix well. Assay Buffer /DTT solution can be stored at - 20°C.

9.3 TEV Protease Dilution Buffer (10 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.4 TEV Protease Positive Control/TEV Protease (Positive Control) (10 µL):

Ready to use as supplied. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at - 80°C.

Immediately prior starting the assay, dilute 5 µL TEV Protease in 45 µL of TEV Protease Dilution Buffer to prepare a 1 IU/µL solution. 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. Keep on ice while in use.

9.5 TEV Protease Substrate (200 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.6 5-FAM Standard/5-FAM Standard (100 µM in DMSO) (100 µL):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time is needed.

Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 10 µM 5-FAM working standard solution by adding 10 µL of 5-FAM Standard/100 µM 5-FAM standard to 90 µL of TEV Protease Assay Buffer.

10.2 Using 10 µM 5-FAM working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	5-FAM 10 µM standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount 5-FAM in well (pmol/well)
1	0	300	100	0
2	6	294	100	20
3	12	288	100	40
4	18	282	100	60
5	24	276	100	80
6	30	270	100	100

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

Δ Note: If the target sample has low protease activity, you will need to use a 10-fold diluted Standard. Prepare a 1 µM standard solution (10 µL of 100 µM standard + 990 µL of TEV Protease Assay Buffer) and set up standard curve dilution as described in the table.
End amount of 5-FAM in the wells will be 0-2-4-6-8-10 pmol/well respectively.

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples at -80°C in TEV Protease Dilution Buffer containing 20% Glycerol.

11.1 TEV-infected cell lysates:

11.1.1 Collect the virus-infected cells by centrifugating them for 10 minutes at 1000 x*g* at 4°C.
11.1.2 Resuspend the pellet in cold PBS.
11.1.3 Lyse the cells by freezing/thawing cycles: freeze at -70°C in dry ice/methanol and thaw at 37°C in water bath for 5 cycles. Vortex for 30 seconds after each cycle.
11.1.4 Spin the cell debris at 1,000 x*g* for 10 minutes.
11.1.5 Collect the supernatant and transfer to a new tube.
11.1.6 Keep on ice.
11.1.7 Measure the amount of the protein in the lysate using BCA Protein Assay Kit (reducing agent compatible).

Δ Note: Sample lysate can be diluted in TEV Protease Dilution Buffer.

11.2 Purified protein:

No sample preparation is required.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Plate Loading:

- Standard wells = 100 µL standard dilutions.
- Positive Control = 10 µL of diluted TEV Protease Positive Control/TEV Protease (adjust volume to 50 µL/well with TEV Protease Dilution Buffer).
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with TEV Protease Dilution Buffer).
- Sample Background Control wells = 1 – 50 µL samples (adjust volume to 100 µL/well with TEV Protease Dilution Buffer).

12.2 TEV Protease Reaction Mix:

12.2.1 Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)
TEV Protease Assay Buffer	48
TEV Protease Substrate	2

12.2.2 Add 50 µL of Reaction Mix into each sample and positive control well. Do NOT add reaction mix to Standard wells or Sample Background Control wells.

12.2.3 Mix well.

12.3 Measurement:

12.3.1 Measure immediately fluorescence at Ex/Em = 490/528 nm in a microplate kinetic mode for 15 - 30 minutes at 34°C.

Δ Note: The assay can be run at temperature s between 22 - 37°C, but the sensitivity will vary accordingly.

Δ Note: Incubation time depends on the TEV activity in the samples. Longer incubation time may be required if activity in the sample is low. If too low, we recommend using a diluted 5-FAM standard (Section 10).

We recommend measuring fluorescence in kinetic mode, and choosing two time points (T_1 and T_2) in the linear range to calculate TEV activity of the samples. The 5-FAM Standard Curve can be read in end point mode (ie. at the end of incubation time assay).

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

13.1.1 Subtract the mean fluorescence value of the blank (Standard #1) from all the standard readings. This is the correct fluorescence.

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of TEV Protease in the sample:

13.2.1 For all reaction wells, choose two points (T_1 and T_2) in the linear phase of the reaction progress curves and obtain the corresponding absorbance values at those points (RFU_1 and RFU_2).

13.2.2 Calculate ΔRFU as follows:

$$\Delta RFU = RFU_2 - RFU_1$$

13.2.3 If significant, subtract the sample background control from the sample reading.

13.2.4 Apply variation of fluorescence in the sample (ΔRFU) to the 5 - FAM Standard Curve to get B pmoles of corresponding product formed during the reaction.

13.2.5 TEV Protease activity (pmol/min/mL) in the test sample is calculated as:

$$TEV\ Protease\ Activity = \frac{B}{\Delta T * V} * D$$

Where:

B = amount of product calculated from the 5-FAM standard curve (pmol)

ΔT = linear phase reaction time $T_2 - T_1$ (min)

V = volume initially added into the reaction well (mL)

D = sample dilution factor.

Alternatively, activity can be displayed based on protein amount (pmol/min/mg) using the following formula:

$$TEV\ Protease\ Activity\ (mg\ protein) = \frac{B}{\Delta T * M} * D$$

Where:

B = amount of product calculated from the 5-FAM standard (pmol).

ΔT = linear phase reaction time $T_2 - T_1$ min)

M = Amount of protein in the sample (mg)

14. Typical Data

Typical standard curve – data provided for **demonstration purposes** only. A new standard curve must be generated for each assay performed.

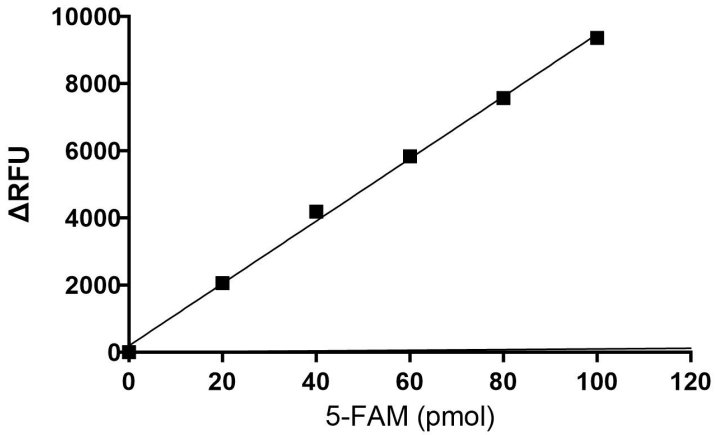


Figure 1: Typical 5-FAM standard curve (20-100 pmol).

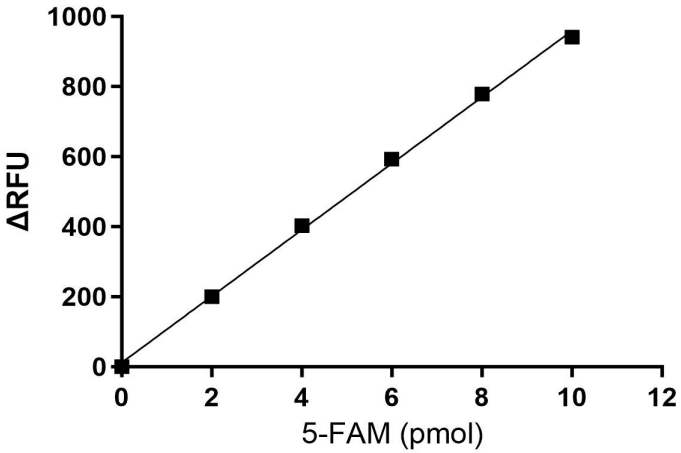


Figure 2: Adjusted 5-FAM Standard curve (2-10 pmol) to be used when sample shows TEV protease activity.

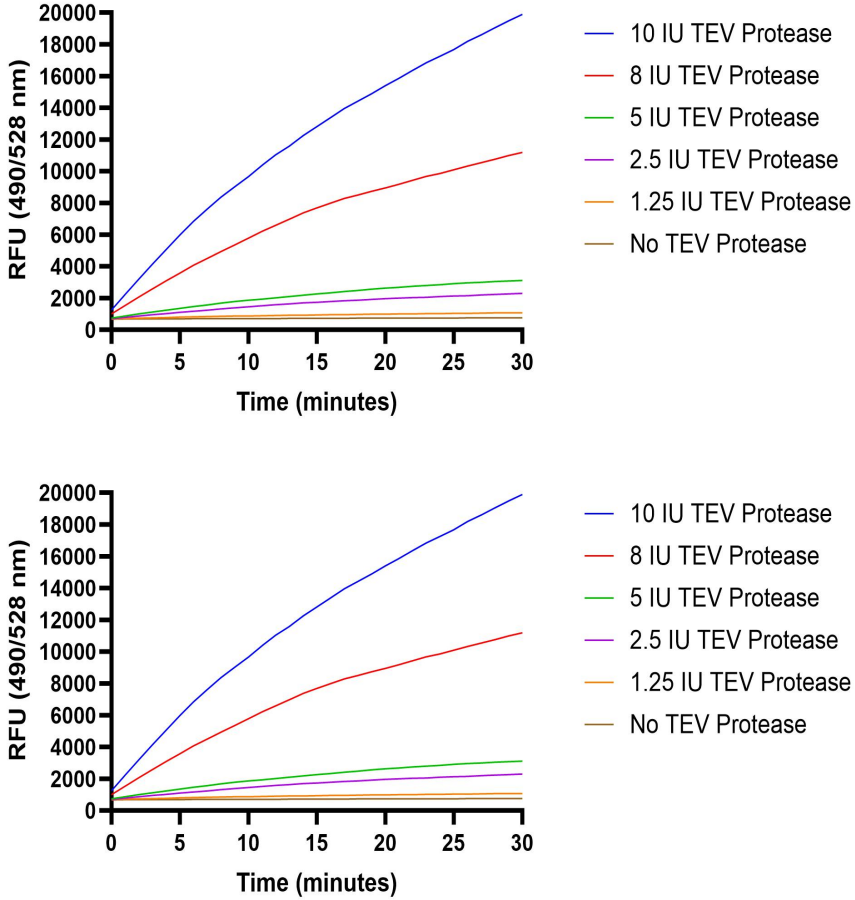


Figure 3. Kinetics progress curves observed for increasing amounts of TEV Protease (positive control included in kit). Assays were performed following the kit protocol.

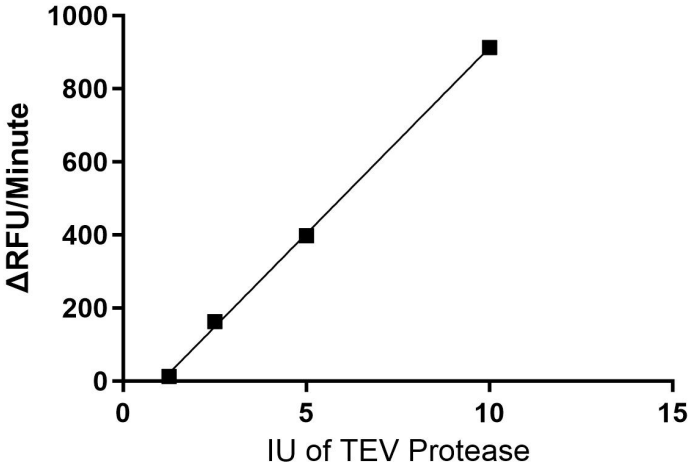


Figure 4. Calculated ΔRFU (after 10 min) for increasing amounts of TEV Protease from kinetic curves shown in figure 3. Assays were performed following the kit protocol.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare TEV Protease standard dilution [20 – 100 pmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (100 µL), sample background control samples (100 µL), sample (50 µL) and positive control wells (50 µL).
- Prepare a master mix for TEV Protease Reaction Mix:

Component	Reaction Mix (µL)
TEV Protease Assay Buffer	48
TEV Protease Substrate	2

- Add 50 µL Reaction mix to sample and positive control wells. DO NOT add Reaction mix to Standard or sample background control wells.
- Measure fluorescence immediately on a microplate reader at Ex/Em= 490/528 nm in kinetic mode at 34°C for 15 – 30 minutes.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

17. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)