SensoLyte® 520 TEV (Tobacco Etch Virus) Protease Assay Kit

Fluorimetric

Catalog #	AS-72227
Kit Size	100 Assays (96-well plate)

- ***Convenient Format:*** Complete kit including all the assay components.
- ***Optimized Performance:*** Optimal conditions for the detection of TEV protease activity.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	5-FAM/QXL™ 520 TEV Substrate, Ex/Em=490 nm/520 nm upon cleavage	50 µL
Component B	5-FAM, Fluorescence Reference Standard, Ex/Em=490 nm/520 nm	100 µM, 10 µL
Component C	TEV Protease, Recombinant	2 µG X 4 vials
Component D	Assay buffer	30 mL
Component E	DTT, 1M	30 µL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat bottom 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of excitation at 490 nm and detecting emission at 520 nm.

Storage and Handling

- Store all kit components at -20°C, except for Component C
- Store Component C at -80°C
- Protect Components A and B from light and moisture
- Component D can be stored at room temperature for convenience

Introduction

Tobacco Etch Virus (TEV) proteinase is widely used to remove fusion tags from recombinant proteins. It recognizes specific sequence Glu-Asn-Leu-Tyr-Phe-Gln-Gly and cleaves between Glutamine and Glycine residues. TEV protease is active even at 4 °C that makes it protease of choice for the fusion proteins or tags removal.[1-3]

The SensoLyte® TEV Protease Assay Kit uses TEV specific Glu-Asn-Leu-Tyr-Phe-Gln-Gly substrate labeled with 5-FAM dye and QXL 520™ quencher. Proteolytic cleavage of this quenched 5-FAM-QXL520™ conjugate yields brightly green fluorescence, which can be continuously monitored at Ex/Em= 490/520 nm. Increase in fluorescence intensity is directly proportional to the TEV protease quantity/activity.

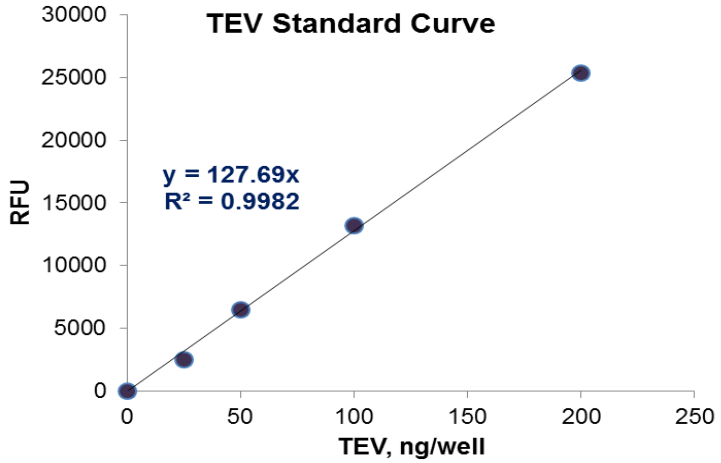

Figure 1. Proteolytic cleavage of 5-FAM-QXL-520 labeled TEV substrate. Fluorescence signal was measured by fluorescence microplate reader (Flexstation 384II. Molecular Devices) with Ex/Em=490 nm/520 nm.

Protocol

Note 1: Warm all kit components to room temperature before starting the experiments.

Assaying TEV protease activity using purified enzyme as a standard.

1. **Prepare working solutions.**

1.1 Prepare assay buffer: Prepare fresh assay buffer for each experiment according to the **Table 1**.

Use this DTT-containing assay buffer in all consecutive steps.

Table 1. Assay buffer for one 96-well plate (100 assays)

Components	Volume
Assay buffer (Component D)	9.99 mL
1 M DTT (Component E)	10 µL
Total volume	10 mL

1.2 Prepare TEV substrate solution: Dilute TEV protease substrate (Component A) 100-

fold in assay buffer (from 1.1). Required amount of protease substrate solution per assay in 96-well plate is 50 µl. Prepare it according to the **Table 2**.

Table 2. Protease substrate solution for one 96-well plate (100 assays).

Components	**Volume**
Protease substrate (100X, Component A)	50 µL
1X Working Assay Buffer	4.95 mL
Total volume	5 mL

Note 1: Dilute substrate according to the amount needed. Save the rest of the 100X substrate stock solution for future experiments.

1.3 Prepare TEV enzyme standards: Prepare TEV standards according to the **Table 3**.

Table 3. TEV standards preparation.

Step	**TEV Concentration, µg/ml**	**TEV (Component C)**	**Assay Buffer**
1	4	Use entire vial (add Assay Buffer directly)	495 µl
2	2	250 µl from Step 1	250 µl
3	1	250 µl from Step 2	250 µl
4	0.5	250 µl from Step 3	250 µl

Note 1: Briefly spin down TEV protease before preparing standards.

2. Set up the enzymatic reaction.

2.1 Add prepared TEV protease standards to the microplate wells: 50 µL of each standard per well. Triplicates are recommended.

2.2 Simultaneously establish the following control wells:

- Substrate control contains 50 µl of assay buffer.

3. Initiate the enzymatic reaction.

3.1 Add 50 µL protease substrate solution to all assayed wells. Mix gently for 30 sec.

3.2 Measure fluorescence signal:

For kinetics reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 minutes for 60 minutes.

For end-point reading: Incubate reaction at the desired temperature for 60 minutes, keep plate away from light. Then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

Appendix I. Data Analysis

- The fluorescence reading from the substrate control well is the background fluorescence. The readings from other wells need to be subtracted with this background fluorescence.
- Plot TEV standards curve (RFU versus TEV enzyme amount). See **Figure 1** for an example.

- Obtain the initial reaction velocity (Vo) in RFU/min. Determine the slope of the linear portion of the data plot. Refer to the Appendix I for the instrument calibration.
- Specific activity of TEV enzyme can be evaluated using reference 5-FAM standard.

Appendix II. Instrument Calibration

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- 5-FAM fluorescence reference standard: Dilute 100 μM 5-FAM (Component B) to 1 μM with assay buffer. Perform 2-fold serial dilutions to get concentrations of 500, 250, 125, 62.5, 31.25, 15.63 and 0 nM. Add 50 μL/well (96-well plate) or 20 μL/well (384-well plate) of serially diluted 5-FAM from 1 μM to 0 nM.
- Add 50 μL/well (96-well plate) of TEV substrate solution (refer to protocol step 1 for preparation).

  Note: TEV substrate solution should be added to the 5-FAM reference standard to correct the fluorescence inner filter effect.
- Measure the fluorescence intensity of the reference standard wells at Ex/Em=490 nm/520 nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot 5-FAM fluorescent reference standard as RFU (relative fluorescent unit) versus concentration as **Figure 2**.

  Note: The final concentration of 5-FAM reference standard is 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 0 nM. This reference standard is used to calibrate the variation of different instruments and different batch of experiments. It is also an indicator of the amount of final product of the HCV NS3/4A protease enzymatic reaction.

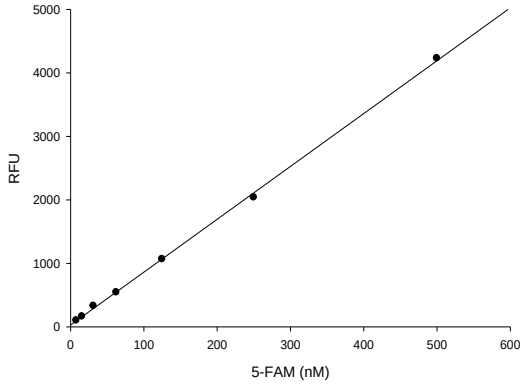

Figure 2. 5-FAM reference standard.

5-FAM was serially diluted in assay buffer containing substrate, and fluorescence was recorded at Ex/Em=490 nm/520 nm. (Flexstation 384II, Molecular Devices)

References

1 Lei Fang, *et.al.*, Prot. Expr. and Pur., **51**, 2006: 102-109
2 Paul G. Blommel, et.al., Prot. Expr. and Pur., **60**, 2007: 1-16
3 L.J.Lucast., et.al., Bio Techniques, **30** (3), 2001: 544-554