EnzChek® Peptidase/Protease Assay Kit (E33758)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light
- Avoid freeze/thaw cycles

Ex/Em: 502/528 nm

Introduction

The EnzChek® Peptidase/Protease Assay Kit (E33758) provides a FRET (fluorescence resonance energy transfer)-based method for the simple and accurate quantitation of a wide range of protease activities. The EnzChek® peptidase/protease substrate comprises a fluorophore and a quencher moiety separated by an amino acid sequence. Upon sequence cleavage by protease(s), the fluorophore separates from the quencher and is free to emit a detectable fluorescent signal (excitation and emission maxima of 502 and 528 nm, respectively). The magnitude of the resultant signal is proportional to the degree of substrate cleavage, and can therefore be used to quantitate the enzyme activity present. The assay is performed in a simple mix–incubate–read format, can be completed in 60 minutes or less, and is easily adaptable to accomodate diverse pH requirements.

Materials

Kit Contents

- **Component A:** EnzChek® peptidase/protease substrate, 1.57 mg
- **Component B:** 20X digestion buffer, 3.5 mL of 200 mM Tris-HCl (pH 7.8)
- **Component C:** Substrate solvent, 1 mL of 50% DMSO in 10 mM Tris-HCl (pH 7.8)

Sufficient materials are supplied for 100 assays, based on a 100 µL assay volume in a 96-well microplate format. The EnzChek® peptidase/protease assay can be adapted for use in cuvettes or 384-well microplates.

Storage

Upon receipt, store the kit at ≤–20ºC, protected from light. Under these conditions the components should be stable for at least 2 years. For short-term storage (days), the buffer (Component B) may be left at room temperature; however, for longer periods we recommend storage at 2–6°C to prevent microbial contamination.

Handling and Disposal

We must caution that no data are available addressing the toxicity of the EnzChek® peptidase/protease substrate. Treat the reagent with the same safety precautions as all other chemicals with unknown toxicity, and dispose of the dye in accordance with local regulations.

Protocol

During all steps, protect the EnzChek® peptidase/protease substrate (both concentrate and working solution) from light as much as possible. Allow the kit components to equilibrate to room temperature prior to use.

1. Prepare a 1X working solution of digestion buffer . For example, to prepare enough 1X digestion buffer for ~20 assays, dilute 0.4 mL Component B into 7.6 mL deionized H_2O. This will make 2 mL of 1X digestion buffer available for substrate preparation and enzyme titration, with 6 mL available for intermediate dilutions. Excess buffer is provided for convenient sample preparation. Store the 1X digestion buffer at 2–6˚C.

2. Make a stock solution of substrate. Add 1 mL of substrate solvent (Component C) to the vial containing the EnzChek® peptidase/protease substrate (Component A). Brief sonication may be necessary. For the best results, substrate stock solution should be used within one week of preparation. Unused stock solution may be stored at ≤–20ºC, but as a result, background signal in the assay may increase over time.

3. Prepare a 1X working solution of the EnzChek® peptidase/protease substrate. For example, for ~20 assays, dilute 0.2 mL of EnzChek® peptidase/protease substrate (Component A) with 0.8 mL of 1X digestion buffer (from Step 1) in a disposable plastic container and mix well. Do not use glass containers. Store the solution at room temperature, protected from light, until ready to use. Table 1 is provided to facilitate the dilution of kit components for a different number of assays; alternatively, the following equations may be used:

A. Buffer Dilution. *volume of 20X buffer concentrate required (mL)* = # assays/50
volume of deionized H_2O required (mL) = (volume of 20X concentrate) × 19

B. Substrate Dilution. *volume of 5X substrate concentrate required (mL)* = # assays/100
volume of 1X buffer required (mL) = (volume of 5X substrate concentrate) × 4

4. Prepare enzyme standard and sample dilutions. Titrate concentrations of enzyme and one buffer-only control. We recommend using at least 0.5 mL of 1X digestion buffer. Detection limits for a wide variety of proteases are given in Table 2.

5. Load microplate wells. Add 50 µL of protease dilutions (from Step 4) into separate wells of a microplate, then add 50 µL of EnzChek® peptidase/protease substrate working solution (from Step 3) and mix well. Duplicates or triplicates are recommended. Incubate for 60 minutes at room temperature, protected from light (incubation times may vary; see "Protocol Details").

6. Measure the fluorescence using a microplate reader. Excitation and emission maxima are 502 and 528 nm, respectively (Figure 1). Excitation/emission settings of 490/520 nm work well in the assay.

7. Use a standard curve to determine protease activity. For the protease standards, plot protease amount vs. fluorescence and fit a line to the data points. Example standard curves are given in Figures 2 and 3.

Protocol Details

Reagent Dilution

Buffers other than that provided may be required for successful use of the EnzChek® peptidase/protease assay. The digestion buffer in this kit is recommended for detecting the protease activity of most proteolytic enzymes with activity optima from pH 7.4 to 8.0. However, if you are working with an enzyme that requires activation compounds or a unique pH environment, prepare the specific buffer required and substitute it for the supplied digestion buffer.

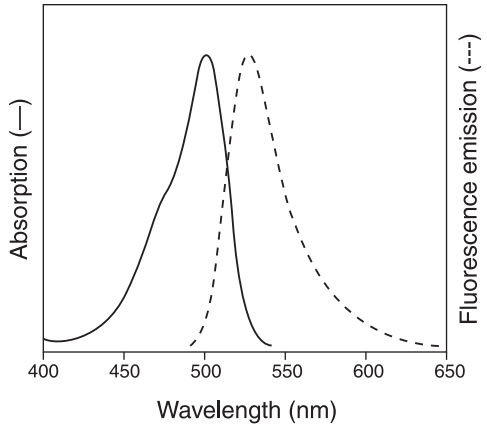

Figure 1. Normalized excitation and emission maxima for the EnzChek® peptidase/protease substrate digestion product in 10 mM Tris-HCl (pH 7.8).

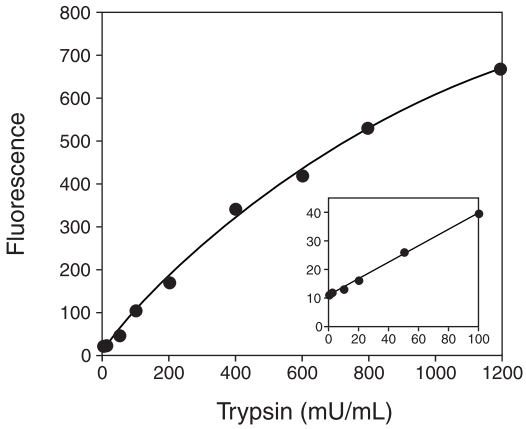

Figure 2. Sample standard curves obtained with the EnzChek® Peptidase/Protease Assay Kit. Trypsin (Sigma T8802, EC 3.4.21.4) was assayed in 10 mM Tris-HCl (pH 7.8) digestion buffer from 10 to 1200 mU/mL using the EnzChek® peptidase/protease substrate. The inset shows a separate experiment using the same enzyme, but at activities from 2 to 100 mU/mL. Samples were incubated for 60 minutes at room temperature. Fluorescence was measured at 490/520 nm; background fluorescence was subtracted from the inset data.

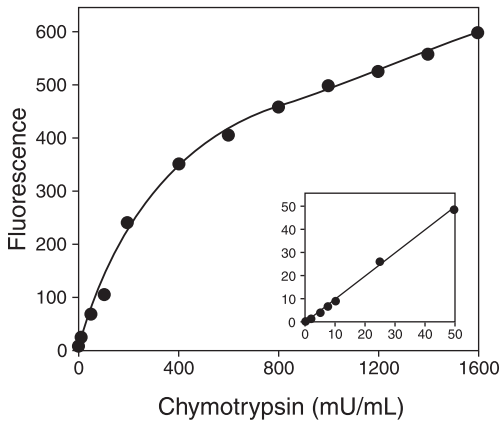

Figure 3. Sample standard curves obtained with the EnzChek® Peptidase/Protease Assay Kit. α-Chymotrypsin (Sigma C7762, EC 3.4.21.1) was assayed from 10 to 1600 mU/mL using the EnzChek® peptidase/protease substrate. The inset shows a separate experiment using the same enzyme, but at activities from 2 to 50 mU/mL. Samples were incubated for 60 minutes at room temperature. Fluorescence was measured at 490/520 nm; background fluorescence was subtracted from the inset data.

Sample Volumes

The assay has been optimized for 100 µL total volume. We recommend preparing enzyme standard and sample dilutions in at least 0.5 mL, then aliquoting into separate microplate wells.

Assay Time and Temperature

The assay temperature is "room temperature," defined here as 20–25°C. Assay temperatures outside of this range have not been tested, but may be acceptable. The assay described here has been optimized for a 60 minute incubation period. Sensitivity may be increased by incubating longer. Conversely, if high sensitivity is not required, incubation times may be reduced. We recommend incubations of at least 5 minutes (Figure 4). The exact time interval is not critical. However, it is important that all reactions be incubated for approximately the same time.

Standard Curves and Detection Limits

Detection limits may vary with instrumentation and enzyme source. Enzyme activity may be affected by incubation buffers and temperature, as well as the storage conditions and number of freeze–thaw cycles to which the enzyme preparation has been subjected. Different dilution schemes of the same enzyme source may also cause variation in activity. For these reasons, we recommend preparing new standard curves on the same day samples are run, preferably on the same microplate. Use an appropriate enzyme standard of known specific activity that closely matches the activity of the enzyme being assayed.

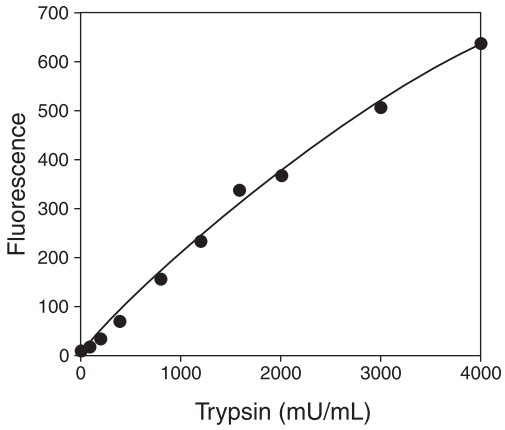

Figure 4. Incubation time with the EnzChek® Peptidase/Protease Assay Kit. Trypsin (Sigma T8802, EC 3.4.21.4) was assayed in 10 mM Tris-HCl (pH 7.8) digestion buffer from 50 to 4000 mU/mL using the EnzChek® peptidase/protease substrate. Samples were incubated for 5 minutes at room temperature and fluorescence was measured at 490/520 nm.

Table 1. Dilutions for the EnzChek® Peptidase/Protease Assay.

	Buffer Dilution			Substrate Dilution	
Number of Assays	**Volume of 20X Buffer (Component B) (mL)**	**Volume of Deionized H_2O (mL)**	**Volume 1X Available (mL)**	**Volume Substrate (Component A) (mL)**	**Volume 1X Buffer (mL)**
10	0.2	3.8	3	0.1	0.4
20	0.4	7.6	6	0.2	0.8
50	1	19	15	0.5	2
100	2	38	30	1	4

Table 2. Detection Limits of the EnzCheck® Peptidase/Protease Assay Kit.

Protease	Source	Detection Limit (mU/mL)
Trypsin from bovine pancreas	Sigma T8802 (EC 3.4.21.4)	2
α-Chymotrypsin (Type I-S) from bovine pancreas	Sigma C7762 (EC 3.4.21.1)	2
Subtilisin A from *Bacillus licheniformis*	Sigma P5380 (EC 3.4.21.62)	0.001
Protease from *Aspergillus oryzae*	Sigma P6110 (EC 3.4.21.63)	0.004
Protease from *Bacillus amyloliquefaciens*	Sigma P1236 (EC 3.4.24.28)	1.0×10^{-5}
Protease from *Bacillus polymyxa*	Sigma P6141 (EC 3.4.24.32)	1.5
Protease from *Bacillus* sp.	Sigma P5985 (EC 3.4.21.62)	0.005
Elastase (Type IV) from porcine pancreas	Sigma E0258 (EC 3.4.21.36)	1
Thermolysin from *Bacillus thermoproteolyticus rokko*	Sigma T7902 (EC 3.4.24.27)	1.5
Papain from *Carica papaya*	Fluka 76218 (EC 3.4.22.2)	0.08
Ficin from fig tree latex	Sigma F6008 (EC 3.4.22.3)	0.04
Bromelain from pineapple stem	Sigma B4882 (EC 3.4.22.32)	0.01
Pepsin from porcine gastric mucosa	Sigma P7000 (EC 3.4.23.1)	0.25

The detection limits listed here are defined as the amount of enzyme required to cause approximately a 10% change in fluorescence compared to a control sample. All samples were incubated for 60 minutes at room temperature using the EnzChek® peptidase/protease assay. Assays were performed in 10 mM Tris-HCl (pH 7.8), except for pepsin, which was assayed in 10 mM HCl (pH 1.8). Dilutions of papain, bromelain, and ficin were made from a buffer containing 30 mM L-cysteine. Enzyme unit definitions are the standard definitions for each individual enzyme. Detection limits may vary; see *Protocol Details*.

Product List *Current prices may be obtained from our website or from our Customer Service Department.*

Cat #	Product Name	Unit Size
E33758	EnzChek® Peptidase/Protease Assay Kit *100 assays*	1 kit

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