Amplite™ Colorimetric Enterokinase Activity Assay Kit *Yellow Color*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Cat#: 11410 (100 Assays)	Keep in freezer and protect from light	Absorbance microplate readers

Introduction

Enterokinase (also called enteropeptidase) is a serine protease produced by cells in the duodenal wall and is a key enzyme in human and animal digestion system. Enterokinase converts trypsinogen into its active form trypsin, resulting in the subsequent activation of pancreatic digestive enzymes. The deficiency of enterokinase results in intestinal digestion impairment. The inhibition of enterokinase may have anti-tumor effects through suppressing proteases involved in carcinogenesis and metastasis. Therefore, highly selective and sensitive detection of enterokinase plays a key role in biochemical applications. Amplite™ Colorimetric Enterokinase Activity Assay Kit offers a sensitive assay for quantifying enterokinase activity. After cleavage of enterokinase, the enterokinase substrate can be detected by EK Yellow™ in an absorbance microplate reader at 405 nm.

Kit Components

Components	**Amount**
Component A: EK Yellow™	1 vial
Component B: Enterokinase Substrate	1 vial
Component C: Assay Buffer	10 mL
Component D: Enterokinase Standard	1 vial
Component E: DMSO	1 vial (100 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare test samples (50 µL) along with serially diluted enterokinase standards (50 µL) → Add equal volume of Assay Mixture (50 µL) → Incubate at 37 °C for 30-60 minutes → Monitor OD increase at 405 nm

Thaw one vial of each kit component at room temperature before starting the experiment.

1. Prepare serially diluted enterokinase standards and test samples:

1.1 Add 50 uL of ddH_2O + 0.1% BSA into Enterokinase Standard vial (**Component D**) to make 10 µg/mL enterokinase stock solution.

1.2 Prepare enterokinase standard dilutions: Add 10 µL of 10 µg/mL enterokinase standard (from step 1.1) into 990 µL of Assay Buffer (**Component C**) to get 100 ng/mL enterokinase solution. Then perform 1:2 serial dilutions in assay buffer to get approximately 50, 25, 12.5, 6.25, 3.13, and 1.56 ng/mL serially diluted enterokinase standards.

1.3 Add enterokinase containing samples and serially diluted enterokinase standards into a 96-well clear bottom microplate according to Tables 1 and 2.

Table 1. Layout of enterokinase standards and test samples in a 96-well clear bottom microplate

BL	BL	TS	TS	….	….						
EK 1	EK 1	….	….	….	….						
EK 2	EK 2										
EK 3	EK 3										
EK 4	EK 4										
EK 5	EK 5										
EK 6	EK 6										
EK 7	EK 7										

Note: EK= Enterokinase Standard, BL=Blank Control (assay buffer), TS=Test Sample.

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Table 2. Reagent composition for each well

EK Standard	Blank Control	Test Sample
Serial Dilutions: 50 µL	Assay Buffer: 50 µL	50 µL

Note 1: Add the serial dilutions of enterokinase standards from 1.56 µM to 100 µM into wells from EK 1 to EK 7.
Note 2: The EK standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity

2. Prepare Enterokinase assay mixture:

2.1 Make EK Yellow™ stock solution (100X):
Add 50 µL of DMSO (**Component E**) into EK Yellow™ (**Component A**) to make 100X stock solution.

2.2 Make Enterokinase Substrate stock solution (100X):
Add 50 µL of DMSO (**Component E**) into Enterokinase Substrate (**Component B**) to make 100X stock solution.

2.3 Make assay mixture:
Add 50 µL of EK Yellow™ stock solution (from Step 2.1) and 50 µL of Enterokinase Substrate stock solution (from Step 2.2) into 5 mL of Assay Buffer (**Component C**), and mix well to make enterokinase assay mixture (Component A+B+C).
Note 1: The assay mixture is enough for one 96-well plate. It is not stable, use it promptly.
Note 2: Store unused EK Yellow™ stock solution at -20ºC, avoid light and repeated freeze-thaw cycles.

3. Run enterokinase assay:

3.1 Add 50 µL of assay mixture (from Step 2.3) into each well of enterokinase standard, blank control, and test samples (see Step 1.3) to make the total assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample, 25 µL of assay mixture into each well.

3.2 Incubate the reaction mixture at 37ºC for 30-60 minutes.

3.3 Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 405 nm.

Data Analysis

The absorbance reading in blank wells (with assay buffer only) is used as a control, and is subtracted from the values of those wells with the enterokinase standards and test samples. The standard curve of enterokinase is shown in Figure 1. Calculate the enterokinase concentrations of the samples according to the standard curve.

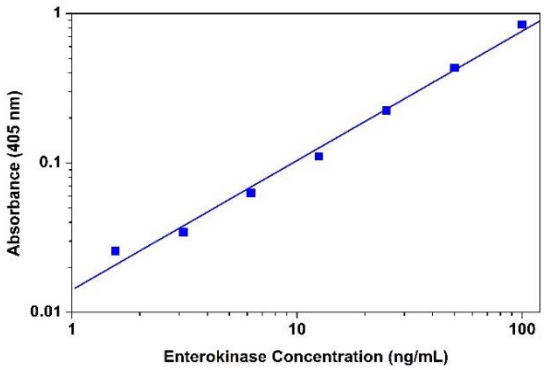

Figure1. Enterokinase dose response was measured with Amplite™ Colorimetric Enterokinase Activity Assay Kit (Cat #11410) on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices) with path check on mode. As low as 3 ng/mL enterokinase was detected with 30 minutes incubation (n=3).

(*Note: The absorbance background increases with time, thus it is important to subtract the absorbance of the blank wells for each data point.)*

References

1. X.L. Zheng, Y. Kitamoto and J.E. Sadler. (2009) Enteropeptidase, a type II transmembrane serine protease. Frontiers in Bioscience E1, pp. 242-249.

2. B. Hadorn, M.J. Tarlow, J.K. Lloyd and O.H. Wolff. (1969) Intestinal enterokinase deficiency. The Lancet, 7599: pp. 812-813.
3. G. Luengo-Gil, M.I. Calvo, E. Martín-Villar, S. Águila, N. Bohdan, A.I. Antón, S. Espín, F.A. de la Peña, V. Vicente, J. Corral, M. Quintanilla and I. Martínez-Martínez. (2016) Antithrombin controls tumor migration, invasion and angiogenesis by inhibition of enteropeptidase. Scientific reports, 27544