Amplite™ Colorimetric Enterokinase Activity Assay Kit

Catalog number: 11410
Unit size: 200 Tests

Component	Storage	Amount
Component A: EK Yellow™	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Enterokinase Substrate	Freeze (<-15 °C), Minimize light exposure	1 vial
Component C: Assay Buffer	Freeze (<-15 °C)	10 mL
Component D: Enterokinase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial
Component E: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

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.

AT A GLANCE

Protocol summary

1. Prepare test samples with diluted enterokinase standards (50 µL)
2. Add equal volume of Enterokinase working solution (50 µL)
3. Incubate at 37 °C for 30 - 60 minutes
4. Monitor OD increase at 405 nm

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

KEY PARAMETERS

Instrument:	Absorbance microplate reader
Absorbance:	405 nm
Recommended plate:	Clear bottom
Instrument specification(s):	Path check on

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

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

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

3. Enterokinase standard solution (10 ug/mL):
Add 50 uL of ddH_2O + 0.1% BSA into Enterokinase Standard vial (Component D) to make 10 µg/mL Enterokinase stock solution.

PREPARATION OF STANDARD SOLUTION

Enterokinase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11410

Add 10 µL of 10 µg/mL Enterokinase standard solution into 990 µL of Assay Buffer (Component C) to get 100 ng/mL enterokinase solution (EK7). Then perform 1:2 serial dilutions in assay buffer to get serially diluted enterokinase standards (EK6 - EK1).

Note The EK standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

PREPARATION OF WORKING SOLUTION

Add 50 µL of EK Yellow™ stock solution and 50 µL of Enterokinase Substrate stock solution into 5 mL of Assay Buffer (Component C); mix well to make Enterokinase (EK) working solution (Component A+B+C).

Note The assay mixture is enough for one 96-well plate. It is not stable, use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of enterokinase standards and test samples in a 96-well clear bottom microplate. EK = enterokinase standard (EK1 - EK7, 1.56 to 100 ng/mL); BL = blank control; TS = test sample.

BL	BL	TS	TS
EK1	EK1	...	...
EK2	EK2	...	...
EK3	EK3		
EK4	EK4		
EK5	EK5		
EK6	EK6		
EK7	EK7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
EK1 - EK7	50 µL	serial dilution (1.56 to 100 ng/mL)
BL	50 µL	Assay Buffer (Component C)
TS	50 µL	sample

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1. Prepare enterokinase standards (EK), blank controls (BL), and test samples (TS) into a 96-well clear bottom microplate according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of EK working solution into each well of enterokinase standard, blank control, and test samples to make the total assay volume of 100 µL/well. For a 384-well plate, add 25 µL of EK working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction mixture at 37 °C for 30 - 60 minutes.

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

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Enterokinase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

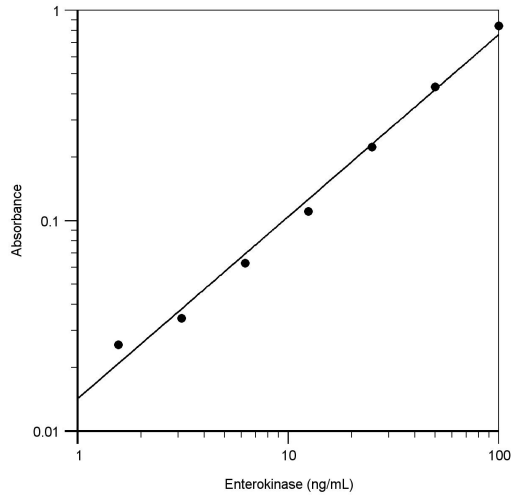

Figure 1. Enterokinase dose response was measured with Amplite™ Colorimetric Enterokinase Activity Assay Kit (Cat #11410) on a 96-well clear bottom microplate using a SpectraMax microplate reader (Molecular Devices) with path check on mode.

DISCLAIMER