ab324118 – Enterokinase Activity Assay Kit (Colorimetric)

A sensitive assay for quantifying enterokinase activity.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab324118

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.

Materials Supplied

Item	Quantity	Storage Condition
EK Yellow™	1 vial	-20°C (Store in the dark)
Enterokinase Substrate	1 vial	-20°C (Store in the dark)
Assay Buffer	1 bottle (10 mL)	-20°C
Enterokinase Standard	1 vial	-20°C (Store in the dark)
DMSO	1 vial (100 µL)	-20°C

Materials Required, Not Supplied

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

- Absorbance microplate reader capable of measuring at 405 nm (Path check on)
- Clear bottom plates
- ddH_2O
- 0.1% BSA

Protocol Summary

1. Prepare test samples and 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 absorbance increase at 405 nm.

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

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.

EK Yellow™ stock solution (100X)

1. Add 50 µL of DMSO into EK Yellow™ to make 100X stock solution.

Enterokinase Substrate stock solution (100X)

1. Add 50 µL of DMSO into Enterokinase Substrate to make 100X stock solution.

Enterokinase standard solution (10 µg/mL)

1. Add 50 µL of ddH_2O + 0.1% BSA into Enterokinase Standard vial to make 10 µg/mL Enterokinase stock solution.

Preparation of Standard Solution

Enterokinase standard

1. Add 10 µL of 10 µg/mL Enterokinase standard solution into 990 µL of Assay Buffer 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

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

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

Experimental Protocol

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

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.

Well	Volume	Reagent
EK1-EK7	50 µL	Serial Dilution (1.56 to 100 ng/mL)
BL	50 µL	Assay Buffer
TS	50 µL	Test Sample

Table 2. Reagent composition for each well of 96-well microplate.

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.

Data Analysis

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.

For technical support contact information, visit: www.abcam.com/contactus

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