ab170964

Enterokinase Activity Assay Kit (Fluorometric)

Instructions for Use

For the accurate measurement of Enterokinase activity in biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 4b, Last Updated 22 April 2025

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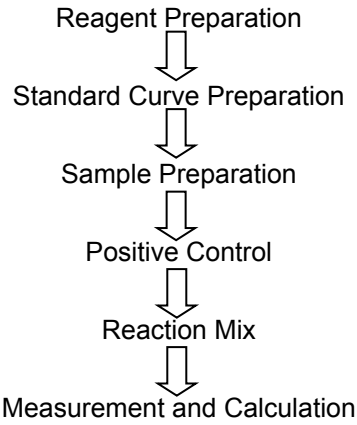
1. Overview

Enterokinase (Enteropeptidase, EC 3.4.21.9) is a serine protease involved in activation of trypsinogen to trypsin, which in turn results in the activation of various digestive enzymes. It recognizes a highly specific amino acid sequence ‘DDDDK’ and cleaves after the lysine residue. High specific activity of Enterokinase has been utilized in cleaving a variety of native or fusion protein tags containing the above recognition motif.

Abcam's Enterokinase Activity Assay Kit (Fluorometric) utilizes a peptide substrate containing the Enterokinase recognition sequence along with a fluorescent label ‘AFC’. Enterokinase catalyzes the cleavage of this substrate and releases the AFC molecule, which can be easily quantified by measuring its fluorescence at Ex/Em = 380/500 nm. This assay kit is simple and rapid and can detect Enterokinase activity as low as 1 µU.

2. Protocol Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
AFC Standard	100 µL	-20°C	-20°C
Enteropeptidase Assay Buffer	20 mL	-20°C	-20°C
Enteropeptidase Substrate	0.2 mL	-20°C	-20°C
Human Enteropeptidase Positive Control/Human Enteropeptidase	50 µL	-20°C	-20°C

4. Storage

Store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm Assay buffer, Enteropeptidase Substrate & AFC Standard to room temperature before use. Briefly centrifuge all small vials at low speed prior to opening (high speed not ideal for enzymes).

5. Additional Materials Required

- 96-well clear plate with flat bottoms (white plates preferred for this assay)
- Multi-well spectrophotometer (ELISA reader)

6. Assay Protocol

A. Reagent Preparation

1. Human Enteropeptidase Positive Control/Human Enteropeptidase (Positive Control):

Ready to use. Aliquot & store at -20°C. Avoid repeated freeze/thaw. Stable for 2 months at -20°C.

B. Enterokinase Assay Protocol

1. AFC Standard Curve:

Dilute AFC Standard to 100 µM (100 pmol/µL) by adding 10 µL of 1 mM AFC Standard to 90 µL Enteropeptidase Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µL of the diluted 100 µM AFC Standard into a series of wells in 96 well plate to generate 0, 200, 400, 600, 800 and 1000 pmol/well of AFC Standard. Adjust the final volume to 100 µL with Enteropeptidase Assay Buffer.

2. **Sample preparation:**

Add 1-50 ul of sample having enteropeptidase activity per well of 96 well plate & add 5-10 µl of Enteropeptidase (Positive Control) into desired well(s). Adjust the final volume to 50 µL with Enteropeptidase Assay Buffer. Prepare in parallel substrate background control well(s) with 50 µL Enteropeptidase Assay Buffer and sample Background Control well(s) with sample + Enteropeptidase Assay Buffer.

3. **Reaction Mix:**

Mix enough reagents for the number of assays to be performed. For each well (Samples, Positive Control & Background Controls), prepare a total 50 µL Reaction Mix containing:

	Reaction Mix	**Background Control Mix**
Enteropeptidase Assay Buffer	48 µL	50 µL
Enteropeptidase Substrate	2 µL	--

Add 50 µl of Reaction mix to the Positive Control, substrate background control & sample wells & 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement**

Incubate the reaction for 30-60 minutes at 37°C. Measure fluorescence at Ex/Em = 380/500 nm in a micro plate reader.

***Note**: Incubation time depends on the Enteropeptidase activity in the samples. We recommend measuring fluorescence in a kinetic mode, and choose two time points (T_1 & T_2) in the linear range to calculate the Enteropeptidase activity of the samples. The AFC Standard Curve can be read in Endpoint mode (i.e. at the end of incubation time).*

7. Data Analysis

Calculation: Subtract the 0 Standard reading from all Standard readings. Plot the AFC Standard Curve. Obtain corrected sample reading by subtracting the substrate background control fluorescence from that of the sample. Calculate the Enteropeptidase activity of the test sample: $\Delta RFU = RFU_2 – RFU_1$.

***Note**: If the sample background control reading is significant, subtract the sample background control fluorescence from that of corrected sample reading.*

Apply the ΔRFU to AFC Standard Curve to get 'B' pmol of AFC generated by Enteropeptidase during the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{Sample Enterokinase activity} = \frac{\textbf{B}}{\Delta\textbf{T}\times\textbf{V}} \times \textbf{Dilution Factor} = \textbf{pmol/min*mL}$$

$$= \textbf{μU/mL}$$

Where:

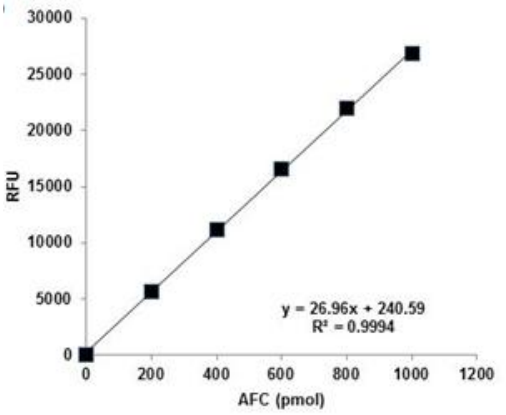
B AFC amount from the Standard curve (pmol)

ΔT is the reaction time (min)

V is the sample volume added into the reaction well (mL)

Unit definition: One unit of Enteropeptidase is the amount of enzyme that generates 1.0 μmol of AFC per min at 37°C.

(a)



(b)

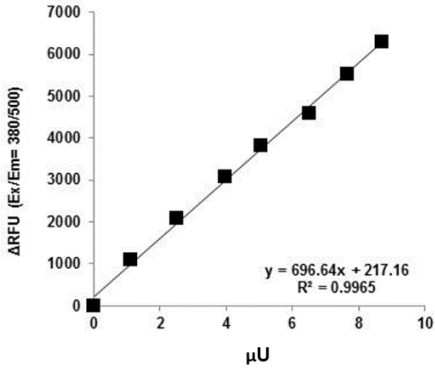


Figure 1: (a) AFC standard curve (b) Human Enteropeptidase Positive Control/Human Enteropeptidase was used to check the sensitivity of the kit. Assays were performed following kit protocol.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)

Problem	Reason	Solution
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix

Problem	Reason	Solution
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)