Enteropeptidase/Enterokinase Activity Fluorometric Assay Kit

rev. 4/13

(Catalog # K758-100; 100 assays; Store kit at -20°C)

I. Introduction:

Enteropeptidase (Enterokinase, 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 Enteropeptidase has been utilized in cleaving a variety of native or fusion protein tags containing the above recognition motif. In Biovision's Enteropeptidase Activity Assay Kit, we have utilized a peptide substrate containing the Enteropeptidase recognition sequence along with a fluorescent label 'AFC'. Enteropeptidase 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 Enteropeptidase activity as low as 1 mU.

II. Application:

- Measurement of Enteropeptidase activity in biological samples or purified Enteropeptidase activity.
- Removing tag from recombinant proteins having recognition motif.

III. Kit Contents:

Components	K758-100	Cap Code	Part Number
Enteropeptidase Assay Buffer	20 ml	WM	K758-100-1
Enteropeptidase Substrate (10 mM, in DMSO)	0.2 ml	Red	K758-100-2
Human Enteropeptidase (Positive Control)	17 µl	Green	K758-100-3
AFC Standard (1 mM)	100 µl	Yellow	K758-100-4

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer (ELISA reader)

V. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer, Enteropeptidase Substrate & AFC Standard to room temperature before use. Briefly centrifuge small vials at low speed (high speed not ideal for enzymes) prior to opening. Read the entire protocol before performing the experiment.

VI. Reagent Preparation and Storage Conditions:

- **Human Enteropeptidase (Positive Control):** Reconstitute with 83 µl Enteropeptidase Assay Buffer. Aliquot & store at -20°C. Avoid repeated freeze/thaw. Stable for 2 months at -20°C.

VII. Enteropeptidase Activity 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 of Positive Control & sample wells 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 50 µl 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 for 30-60 min at room temperature and measure fluorescence at Ex/Em = 380/500 nm.

   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).

5. **Calculations:** 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 ($\Delta T = T_2 - T_1$).

$$\textbf{Sample Enterokinase Activity} = \frac{\boldsymbol{B}}{\Delta \mathbf{T} \times \mathbf{V}} \times \boldsymbol{Dilution\ Factor} = \boldsymbol{pmol min^{-1} ml^{-1}} = \boldsymbol{mU ml^{-1}}$$

Where: **B** is the AFC amount from the Standard Curve (pmol).
Δ**T** 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 nmol of AFC per min at room temperature.

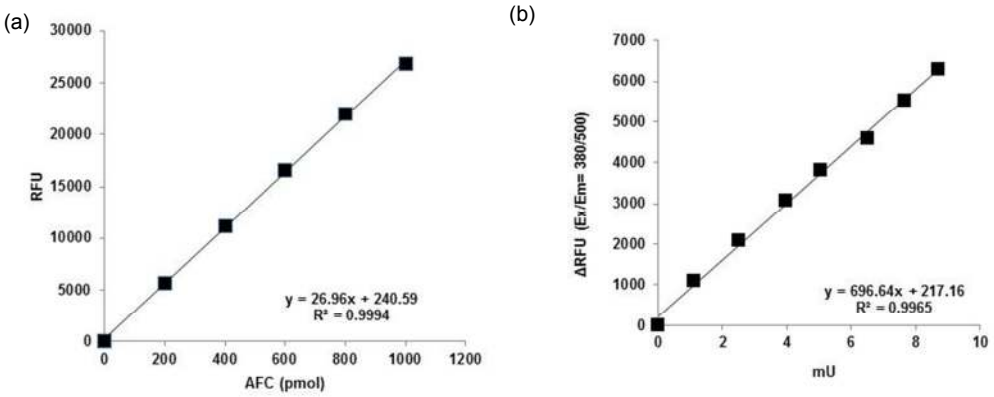

Figure: AFC Standard Curve (a). Human Enteropeptidase was used to check the sensitivity of the kit (b). Assays were performed following kit protocol.

VI. RELATED PRODUCTS:

Trypsin Activity Assay Kit
Asparaginase Activity Assay Kit
Granzyme B Activity Assay Kit
Granzyme B Inhibitor Screening Kit
Lipase Activity Assay Kit
Lipase Activity Assay Kit II
Lipase Activity Assay Kit III
Protease Activity Assay Kit
Endothelial lipase antibody
Endothelial lipase blocking peptide
Protease Inhibitor Cocktail
EZBlock™ Protease Inhibitor Cocktail
EZBlock™ Protease Inhibitor Cocktail, EDTA-Free
EZBlock™ Universal Protease and Phosphatase Inhibitor Cocktail
EZBlock™ Universal Protease and Phosphatase Inhibitor Cocktail, EDTA-Free

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