

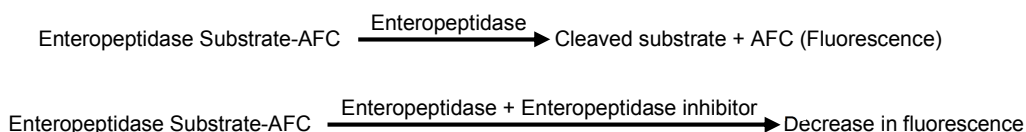
Enteropeptidase/Enterokinase Inhibitor Screening Kit (Fluorometric)

rev. 3/13

(Catalog # K759-100; 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 proteins containing the above recognition. In Biovision's Enteropeptidase inhibitor screening 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. In the presence of potent Enteropeptidase inhibitor, the hydrolyzation of the substrate will be impeded. The kit provides a rapid, simple & reliable test for screening potential inhibitors of Enteropeptidase.



II. Application:

- Screening potential inhibitors of Enteropeptidase
- Characterize/study Enteropeptidase inhibitors

III. Kit Contents:

| Components | K759-100 | Cap Code | Part Number |
|---|----------|----------|-------------|
| Enteropeptidase Assay Buffer | 20 ml | WM | K759-100-1 |
| Enteropeptidase Substrate | 0.2 ml | Red | K759-100-2 |
| Human Enteropeptidase | 0.17 ml | Green | K759-100-3 |
| Enteropeptidase-specific Inhibitor [Aprotinin] (0.6 mM) | 0.1 ml | Blue | K759-100-4 |

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 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: Reconstitute with 910 μ l Enteropeptidase Assay Buffer to obtain a solution of 1mU/ μ l. Aliquot & store at -80°C. Avoid repeated freeze/thaw. Stable for 2 months at -80°C.

VII. Enteropeptidase Inhibitor Screening Protocol:

1. Enteropeptidase Enzyme Preparation: For each well, prepare 50 μ l of Enteropeptidase enzyme solution.

40 μ l Enteropeptidase Assay Buffer
10 μ l Enteropeptidase enzyme (1mU/ μ l)

2. Screening compounds, inhibitor control & blank control preparations: Dissolve candidate inhibitors into proper solvent. Dilute to 4X the desired test concentration with Enteropeptidase Assay Buffer. Add 25 μ l diluted test inhibitors or Assay Buffer into Enteropeptidase enzyme wells as sample screen [S] or Enzyme Control [EC] (no inhibitor). For Inhibitor Control, add 10 μ l Enteropeptidase-specific inhibitor & 15 μ l Enteropeptidase Assay Buffer to Enteropeptidase enzyme well(s). Incubate at room temperature for 10-15 min.

3. Enteropeptidase Substrate Preparation: For each well, prepare 25 μ l of the substrate solution.

23 μ l of Enteropeptidase Assay Buffer
2 μ l of Enteropeptidase Substrate

Mix & add 25 μ l of Enteropeptidase Substrate solution into each well. Mix well.

4. Measurement: Measure the fluorescence in a kinetic mode for 30-60 min (Ex/Em = 380/500 nm). Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU₁ and RFU₂).

5. Calculations: Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net Δ RFU (RFU₂-RFU₁) values with the time ΔT (T_2 - T_1).

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of EC} - \text{Slope of Sample}}{\text{Slope of EC}} \times 100$$

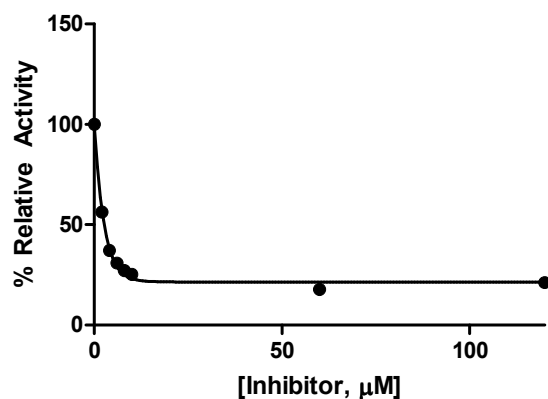


Figure: Inhibition of Enteropeptidase activity by Enteropeptidase-specific Inhibitor, Aprotinin. Assay was performed following kit protocol.

VIII. RELATED PRODUCTS:

Enteropeptidase Activity Fluorometric Assay Kit
 Granzyme B Activity Fluorometric Assay Kit
 Granzyme B Inhibitor Screening Kit (Fluorometric)
 Human Calpain 1 Inhibitor Screening Kit (Fluorometric)
 DPP4 Inhibitor Screening Kit (Fluorometric)
 HDAC Inhibitor Drug Screening Kit (Fluorometric)
 HDAC-3 Inhibitor Drug Screening Kit (Fluorometric)
 HDAC-8 Inhibitor Drug Screening Kit (Fluorometric)
 MMP-1 Inhibitor Screening Kit
 MMP-3 Inhibitor Screening Kit
 TACE inhibitor Screening Assay Kit (Fluorometric)
 CETP inhibitor Drug Screening Kit (Fluorometric)
 PLTP inhibitor Drug Screening Kit (Fluorometric)

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