Technical Bulletin

Enterokinase (Enteropeptidase) Activity Assay Kit

Catalogue number MAK544

Product Description

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.

The Enterokinase Activity Assay Kit offers a sensitive assay for quantifying enterokinase activity. After cleavage by enterokinase, the enterokinase substrate can be detected by Enterokinase Detection Reagent in an absorbance microplate reader at 405 nm.

Components

The kit is sufficient for 200 colorimetric assays in 96-well plates.

- Enterokinase Detection Reagent 1 Vial
  Catalogue Number MAK544A
- Enterokinase Substrate 1 vial
  Catalogue Number MAK544B
- Assay Buffer 10 mL
  Catalogue Number MAK544C
- Enterokinase Standard 1 Vial
  Catalogue Number MAK544D
- DMSO 100 µL
  Catalogue Number MAK544E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Bovine Serum Albumin (Catalogue Number A7030 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use.

Procedure

All samples and standards should be run in duplicates.

Preparation of Stock Solution

Enterokinase Detection Reagent stock solution (100X): Add 50 µL of DMSO into Enterokinase Detection Reagent to make 100X stock solution.

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

Enterokinase Standard Solution (10 µg/mL): Add 50 µL of purified H_2O + 0.1% BSA into Enterokinase Standard vial to make 10 µg/mL Enterokinase stock solution.

Preparation of Standard Curve

Note: Fresh reconstitution of the Standard Solution is recommended.

1. Enterokinase Standard: Add 10 µL of 10 µg/mL Enterokinase Standard solution into 990 µL of Assay Buffer to get 100 ng/mL Enterokinase solution (EK1).
2. Perform 1:2 serial dilutions in Assay Buffer to get serially diluted Enterokinase Standards (EK2 – EK7) as shown in Table 1.

Table 1.

Serial Dilution of Enterokinase Standard

Dilution	EK Standard Volume (µL)	Serial Dilution Source	Assay Buffer Volume (µL)	Conc (ng/mL)
EK1	300	from 100 ng/mL stock	0	100
EK2	150	From EK1	150	50
EK3	150	From EK2	150	25
EK4	150	From EK3	150	12.5
EK5	150	From EK4	150	6.25
EK6	150	From EK5	150	3.125
EK7	150	From EK6	150	1.5625

Preparation of Enterokinase Working Solution

Combine 50 µL of Enterokinase Detection Reagent stock solution and 50 µL of Enterokinase Substrate stock solution into 5 mL of Assay Buffer. Mix well.

Assay Reaction

Note: Use Assay Buffer for the blank wells.

1. Add 50 µL of each standard, test sample, and blank into separate wells of a 96-well clear bottom plate.
2. Add 50 µL of Enterokinase Working Solution into each well containing standard, blank, and test sample to make the total assay volume of 100 µL/well.

Measurement

1. Incubate the reaction mixture at 37 °C for 30 - 60 minutes.
2. Monitor the absorbance increase with an absorbance plate reader with path check on at OD of 405 nm.

Results

1. The absorbance obtained from the blank standard well is used as a negative control.
2. Subtract the blank value from the standards to obtain the base-line corrected values.
3. Plot the standards readings to obtain a standard curve and equation. The equation can be used to calculate Enterokinase concentration of the samples.

Convert concentration to activity:

$$\boldsymbol{Conc.} = \frac{\Delta \boldsymbol{A}}{\boldsymbol{\varepsilon l}} = \mu\mathbf{M}$$

$$\mu\frac{\mathbf{M}}{\mathbf{min}} = \frac{\mu\frac{\mathbf{moles}}{\mathbf{L}}}{\mathbf{min}} = \frac{\mu\frac{\mathbf{moles}}{\mathbf{min}}}{\mathbf{L}} = \frac{\boldsymbol{U}}{\boldsymbol{L}} = \frac{\boldsymbol{mU}}{\boldsymbol{mL}}$$

Where:

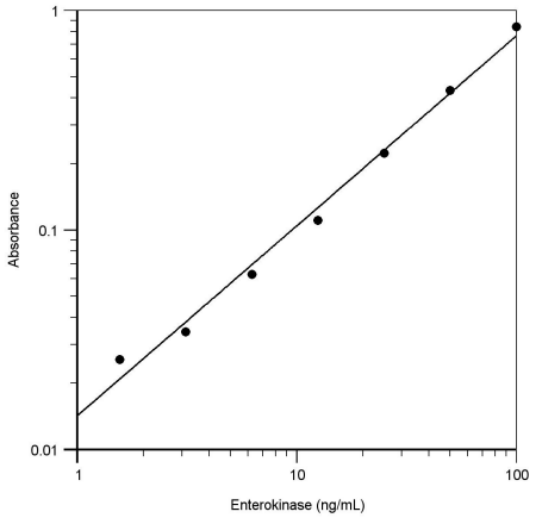
ε = molar extinction coefficient = 14200 $M^{-1}cm^{-1}$

l = pathlength (cm)

ΔA = Change in absorbance (value from step 2)

Figure 1.

Typical Enterokinase Standard Curve



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