Technical Bulletin

Enteropeptidase/Enterokinase Activity Assay Kit

Catalog Number MAK204

Product Description

Enteropeptidase (Enterokinase; EC 3.4.21.9) is an enzyme that converts trypsinogen to trypsin, the activity of which is required for the activation of chymotrypsin and procarboxypeptidases. It has an important role in food digestion and may serve as a marker of differentiated enterocytes and goblet cells.[1,2]

The Enteropeptidase/Enterokinase Activity Assay Kit is a rapid method to measure enteropeptidase activity in biological samples. Enteropeptidase activity is measured by cleaving a synthetic 7-amino-4-trifluoromethylcoumarin (AFC)-tagged peptide substrate containing the recognition sequence for enteropeptidase. This yields AFC, a fluorescent product (λ_{Ex} = 380 nm/ λ_{Em} = 500 nm), proportional to the enzymatic activity present.

Components

The kit is sufficient for 100 fluorescent assays in 96-well plates.

- Enteropeptidase Assay Buffer 20 mL
  Catalog Number MAK204A
- Enteropeptidase Substrate, 10 mM in DMSO 0.2 mL
  Catalog Number MAK204B
- Human Enteropeptidase 50 µL
  Catalog Number MAK204C
- AFC Standard, 1 mM 100 µL
  Catalog Number MAK204D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.

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, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Enteropeptidase Assay Buffer, Enteropeptidase Substrate, and AFC Standard: Warm each to room temperature before use.

Human Enteropeptidase (Positive Control): Ready to use. Aliquot and store at –20 °C. Use within 2 months after aliquoting.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Add 1–50 µL of the Sample into duplicate wells. Bring Samples to a final volume of 50 µL using Enteropeptidase Assay Buffer.

   Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the Standard curve.

2. For samples exhibiting significant background, include a Sample Background Control for each Sample by omitting the Enteropeptidase Substrate. The Sample Background Control readings can then be subtracted from the Sample readings.
3. For a Positive Control (optional), add 5-10 µL of the Human Enteropeptidase (Positive Control) solution to the desired wells. Adjust the final volume to 50 µL with Enteropeptidase Assay Buffer.

AFC Standards for Fluorometric Detection

1. Prepare a 100 µM AFC Standard Solution by diluting 10 µL of the 1 mM (1 nmol/µL) AFC Standard Solution with 90 µL of Enteropeptidase Assay Buffer.
2. Prepare AFC Standards by adding the volumes of 100 µM (100 pmol/µL) AFC Standard Solution and Enteropeptidase Assay buffer into wells of the plate according to Table 1.

Table 1.

Preparation of Fluorometric AFC Standards

Well	100 µM AFC Standard	Entero-peptidase Assay Buffer	AFC (pmol)
1	10 µL	90 µL	1000
2	8 µL	92 µL	800
3	6 µL	94 µL	600
4	4 µL	96 µL	400
5	2 µL	98 µL	200
6	--	100 µL	0

Assay Reaction

1. Prepare Substrate Background Controls by adding 50 µL of Enteropeptidase Assay Buffer to duplicate wells.
2. Set up the Reaction Mix according to the scheme in Table 2. 50 µL of the appropriate Reaction Mix is required for each Sample, Positive Control, and Substrate Background Control reaction (well).

Table 2.

Preparation of Reaction Mix

Reagent	Reaction Mix
Enteropeptidase Assay Buffer	48 µL
Enteropeptidase Substrate	2 µL

3. Add 50 µL Enteropeptidase Assay Buffer to each of the Sample Background Control wells.
4. Add 50 µL of Reaction Mix to each of the Sample, Positive Control, and Substrate Background Control wells.
5. Mix plate well using a horizontal shaker or by pipetting.
6. Incubate the plate at 37 °C. Measure the fluorescence (RFU, λ_{Ex} = 380 nm/ λ_{Em} = 500 nm) in a microplate reader in kinetic mode for 30-60 minutes at 37 °C. Protect the plate from light during the incubation. It is recommended to take fluorescent readings every minute.

   Note: Incubation time depends on the activity of enteropeptidase in the samples.

   Note: The AFC Standards can be read at the end of the incubation time.

Results

Calculations

1. Plot the fluorescence (RFU) for each Sample and Sample Blank well versus time.
2. Choose two time points (T_1 and T_2) in the linear range of the plot and determine the RFU at each time (RFU_1 and RFU_2).

   Note: It is essential that RFU_1 and RFU_2 fall within the linear range of the Standard curve.

4. Subtract the RFU measurement obtained for the 0 AFC Standard (Well 6) from all Standard RFU values. Background values can be significant and must be subtracted from all readings.
5. Use the corrected AFC Standard RFU values to plot the standard curve.

   Note: A new Standard curve must be generated each time the assay is run.

6. Obtain the corrected Sample and Sample Background Control RFU readings by subtracting the Substrate Background Control RFU reading from each of the Sample and Sample Background Control readings. Calculate the change in fluorescence measurement from T_1 to T_2 for the Samples.

   $\Delta RFU = RFU_2 - RFU_1$

7. Subtract the Sample Background Control ΔRFU value from the Sample ΔRFU value to obtain the corrected Sample ΔRFU value.
8. Using the corrected Sample ΔRFU value, determine the amount of AFC (pmole/well) generated by the enteropeptidase assay between T_1 and T_2 (B).
9. Calculate Enteropeptidase activity using the following formula:

   Enteropeptidase Activity (pmole/min/mL or milliunit/mL) =

$$\frac{B}{(\Delta T) \times V} \times DF$$

where:

B = Amount of AFC (pmole) generated in unknown Sample well between T_1 and T_2 from standard curve

ΔT = Reaction Time ($T_2 - T_1$) (minutes)

V = Sample volume (mL) added to well

DF = Dilution factor. DF = 1 for undiluted samples

Unit definition: One unit of enteropeptidase is the amount of enzyme that generates 1.0 μmole of AFC per minute at 37 °C.

Figure 1.

Typical AFC Standard Curve

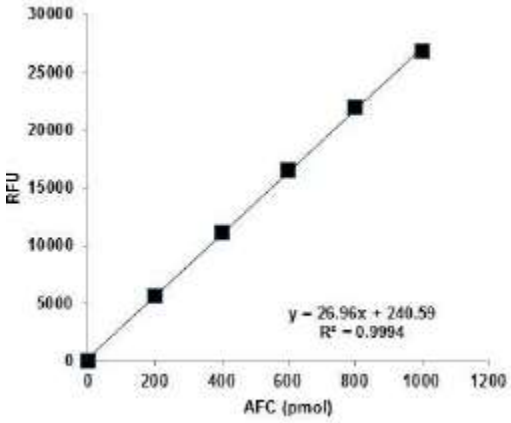
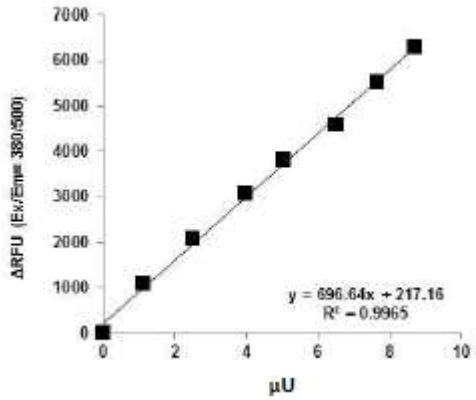


Figure 2.

Human enteropeptidase was used to check the sensitivity of the kit. Assays were performed following kit protocol.



References

1. Imamura, T., and Kitamoto, Y., Expression of enteropeptidase in differentiated enterocytes, goblet cells, and the tumor cells in human duodenum. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **285**, G1235–1241 (2003).
2. Braud, S. et al., Enteropeptidase: a gene associated with a starvation human phenotype and a novel target for obesity treatment. *PLoS One*, **7**, e49612 (2012). doi: 10.1371/journal.pone.0049612.

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