Product Information

Enteropeptidase/Enterokinase Activity Assay Kit

Catalog Number **MAK204**
Storage Temperature –20 °C

**TECHNICAL BULLETIN**

**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<sub>ex</sub> = 380/λ<sub>em</sub> = 500 nm), proportional to the enzymatic activity present. One unit of enteropeptidase is the amount of enzyme that generates 1.0 nmole of AFC per minute at room temperature.

**Components**

The kit is sufficient for 100 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** 17 µL
  Catalog Number MAK204C

- **AFC Standard, 1 mM** 100 µL
  Catalog Number MAK204D

**Reagents and Equipment Required but Not Provided**

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

**Precautions and Disclaimer**

This product is 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.

**Preparation Instructions**

Briefly centrifuge vials at low speed before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

**Procedure**

**AFC Standards for Fluorometric Detection**

Dilute 10 µL of the 1 mM (1 nmole/µL) AFC Standard Solution with 90 µL of Enteropeptidase Assay Buffer to prepare a 100 µM (100 pmole/µL) AFC Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 100 µM (100 pmole/µL) AFC Standard Solution into 96 well plate, generating 0 (blank), 200, 400, 600, 800, and 1,000 pmole/well standards. Add Enteropeptidase Assay Buffer to each well to bring the volume to 100 µL.

**Storage/Stability**

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.
Sample Preparation
All samples and standards should be run in duplicate.

Add 5–50 µL of the samples 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.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the Enteropeptidase Substrate. The Sample Blank readings can then be subtracted from the sample readings.

For a positive control (optional), add 5–10 µL of the Enteropeptidase Positive Control solution to the desired wells. Adjust the final volume to 50 µL with Enteropeptidase Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1. Reaction Mixes

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<th>Reagent</th>
<th>Standards, Controls, and Samples</th>
<th>Sample Blank</th>
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<tr>
<td>Enteropeptidase Assay Buffer</td>
<td>48 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Enteropeptidase Substrate</td>
<td>2 µL</td>
<td>–</td>
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2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Measure the fluorescence (FLU, $\lambda_{ex} = 380$/ $\lambda_{em} = 500$ nm) in a microplate reader in kinetic mode for 30–60 minutes at room temperature. 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.

4. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (1,000 pmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

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

Calculations

Plot the fluorescence (FLU) for each well versus time.

Choose two time points (T1 and T2) in the linear range of the plot and determine the FLU at each time (FLU1 and FLU2).

Note: It is essential that FLU1 and FLU2 fall within the linear range of the standard curve.

Correct for the background by subtracting the measurement obtained for the blank AFC standard from that of the standards, controls, and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate AFC Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in fluorescence measurement from T1 to T2 for the samples.

\[ \Delta \text{FLU} = \text{FLU}_2 - \text{FLU}_1 \]

Subtract the Sample Blank \( \Delta \text{FLU} \) value from the Sample \( \Delta \text{FLU} \) reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of AFC (pmole/well) generated by the enteropeptidase assay between T1 and T2 (\( S_a \)).

Enteropeptidase activity:

Enteropeptidase Activity = \( \frac{S_a}{(\text{Reaction Time}) \times S_v} \)

where:

- \( S_a \) = Amount of AFC (pmole) generated in unknown sample well between T1 and T2 from standard curve
- Reaction Time = T2 – T1 (minutes)
- \( S_v \) = sample volume (mL) added to well

Enteropeptidase activity is reported as pmole/min/mL = milliunit/mL.

One unit of enteropeptidase is the amount of enzyme that generates 1.0 nmole of AFC per minute at room temperature.

Sample Calculation:

Amount of AFC (\( S_a \)) = 584 pmole (from standard curve)

(T1) = 3 minutes

(T2) = 32 minutes

Sample volume (\( S_v \)) = 0.050 mL

Enteropeptidase activity in sample well:

\[ \text{pmole/min/mL} = \frac{584 \text{ pmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 0.050 \text{ mL/well}} = 403 \text{ (milliunits/mL)} \]

References


# Troubleshooting Guide

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<td>Prepare fresh Reaction Mixes before each use</td>
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<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
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