# Enterokinase Activity Assay Kit (100 T)

**Cat. No.** KITG-037 **Lot. No.** (See product label)

## SPECIFICATION

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| **Product Overview** | Enterokinase (EC 3.4.21.9) is a serine protease that specifically recognizes the sequence (Asp)4-Lys and cleaves the peptide bond after the lysine residue. It plays a key role in the digestive system by activating trypsinogen to trypsin, which subsequently activates other zymogens. Its expression is specifically localized to differentiated intestinal cells and goblet cells, making it both a core regulator of nutrient digestion and a marker for intestinal disease research. This assay kit utilizes a 7-amino-4-trifluoromethylcoumarin (AFC)-labeled peptide substrate to detect enterokinase activity. The fluorescent product AFC generated by the reaction (Excitation wavelength λEx=380 nm, Emission wavelength λEm=500 nm) can be quantified by a fluorescence microplate reader to calculate enterokinase activity. |
| **Description** | This Enterokinase Activity Assay Kit provides a convenient and sensitive fluorescence-based method for measuring enterokinase activity. The kit uses an AFC-labeled peptide substrate that is specifically cleaved by enterokinase to release free AFC, producing a strong fluorescent signal. The assay is suitable for purified enterokinase samples and can detect enzyme activity in the range of 2-50 mU with high sensitivity. The kit includes all necessary components for performing the assay and generating a standard curve for quantification. This kit is not suitable for testing crude extracts. |
| **Form** | Liquid |
| **Applications** | Measurement of enterokinase activity in purified enzyme preparations; Screening and characterization of enterokinase inhibitors; Quality control of enterokinase production; |

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| | Research on digestive enzyme activation mechanisms and intestinal disease markers |
| **Notes** | This kit is not suitable for testing crude extracts. For samples with unknown enzyme activity, prepare multiple dilution gradients to ensure the fluorescence change falls within the linear range of the standard curve (optimal enzyme input: 2-50 mU). All components should be equilibrated to room temperature (15-30 min) before use. Mix each component gently by vortexing, then centrifuge briefly (5000 rpm, 1 min) before use. The substrate and AFC standard should be protected from light. All samples and standards should be measured in duplicate to reduce experimental error. Transfer enzyme samples using pre-wetted tips to avoid adsorption losses. Read immediately after adding enzyme; do not delay. Wear lab coat and disposable gloves for operation. For research use only. |
| **Storage** | Store at -25 to -15°C. Valid for 6 months after opening. |
| **Shipping** | Shipped with dry ice or ice packs |
| **Reaction conditions** | Reaction temperature: 25°C Reaction time: 30 min (can be extended to 60 min for low-activity samples) Excitation wavelength: 380 nm Emission wavelength: 500 nm Microplate reader settings: Fluorescence mode, Kinetic reading, 96-well standard opaque plate, Auto Cutoff, duration 30 min, interval 1 min, 31 intervals, minimum interval 0, PMT and Optics set to Low |
| **EC** | 3.4.21.9 |
| **Unit definition** | One unit is defined as the amount of enzyme that catalyzes the generation of 1 nmol of AFC per minute at 25°C. |
| **Usage** | Kit Components (100 Tests): 200× Enterokinase Substrate: 24 µL 1 mM AFC Standard: 100 µL Enterokinase Assay Buffer: 60 mL Materials Required but Not |

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Provided: Pipettes and tips of various specifications Vortex mixer Benchtop centrifuge White flat-bottom 96-well microplate Amber centrifuge tubes (500 µL / 1.5 mL) Reagent Preparation: 1. Remove all components from storage and allow to equilibrate to room temperature (15-30 min). 2. Mix each component gently by vortexing, then centrifuge briefly (5000 rpm, 1 min) to collect contents at the bottom of the tube. 3. Prepare 1× Enterokinase Substrate by diluting the 200× stock with Enterokinase Assay Buffer. Sample Preparation: 1. This kit is suitable for detecting purified enterokinase samples. Dilute samples with Enterokinase Assay Buffer to appropriate concentrations. Recommended enzyme input: 2-50 mU. Use low-retention tips to avoid sample adsorption losses. 2. For enzyme samples with unknown activity, prepare multiple dilution gradients to ensure readings fall within the linear range of the standard curve. Limit dilution steps to 1-2 steps to minimize dilution errors. 3. After each addition, perform a brief vortex mixing (1 second, 2 times) to avoid excessive shaking that may cause activity loss. 4. Optional Positive Control: Dilute purified enterokinase (5 U/µL) with Assay Buffer to prepare working solutions. Example: Take 2 µL of 5 U/µL enterokinase, add 98 µL Enterokinase Assay Buffer, vortex briefly (1 sec, 2 times) to obtain 0.1 U/µL intermediate dilution. Then take 2 µL of the 0.1 U/µL intermediate dilution, add 198 µL Enterokinase Assay Buffer, vortex briefly again to obtain 0.001 U/µL (1 mU/µL) positive control working solution. Standard Curve Preparation: 1. Prepare 100 µM (100 pmol/µL) AFC Standard solution: Mix 4 µL of 1 mM AFC Standard with 36 µL of Enterokinase Assay Buffer. Vortex to mix, keep at room temperature, protected from light. 2. Prepare AFC standard dilutions in a white 96-well plate (add Buffer first, then AFC standard solution with fresh tips): - 1000 pmol: 10 µL 100 µM AFC + 90 µL Buffer - 800 pmol: 8 µL 100 µM AFC + 92 µL Buffer - 600 pmol: 6 µL 100 µM AFC + 94 µL Buffer - 400 pmol: 4 µL 100 µM AFC + 96 µL Buffer - 200 pmol: 2 µL 100 µM AFC + 98 µL Buffer - 0 pmol: 0 µL 100 µM AFC + 100 µL Buffer 3. Measure fluorescence using Endpoint mode (Ex=380 nm, Em=500 nm, Auto Cutoff, PMT and Optics set to Low). 4. Record the fluorescence value of each well. Assay Procedure: 1. Set up the microplate

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reader: Fluorescence mode, Kinetic reading, 96-well standard opaque plate, Ex=380 nm, Em=500 nm, Auto Cutoff, duration 30 min, interval 1 min, 31 intervals, minimum interval 0, PMT and Optics set to Low. 2. Prepare reaction mixtures in a white flat-bottom 96-well plate (total volume 100 µL per well): - For known activity samples (1 mU/µL): 50 µL Assay Buffer + 10 µL sample + 40 µL 1× Substrate - For multiple dilution samples: (60-x) µL Assay Buffer + x µL sample + 40 µL 1× Substrate 3. Mix gently and immediately place the plate in the microplate reader. 4. Shake for 10 seconds, then start the detection program. 5. Record fluorescence at 25°C for 30 minutes. Important Notes for Assay: - Read immediately after adding enzyme; do not delay as the reaction rate is fast. - Detection time depends on sample activity; extend to 60 min for low-activity samples or increase enzyme input. - AFC standard can be read separately after sample detection; no need to read simultaneously. - The AFC standard curve slope should be within 38±3, with $R^2 \geq 0.99$ when using SpectraMax M3. Different microplate reader brands/models may yield different absolute values; use the same instrument for all samples in one batch to ensure data comparability. Data Analysis: 1. Plot standard curve: AFC amount (pmol) on X-axis, RFU on Y-axis. Use linear regression. Prepare a fresh standard curve for each run. 2. For each sample, plot fluorescence vs. time curve. Select the linear range, with T1 and T2 as the start and end times of the linear range (recommended T1=2 min). Record fluorescence values FU1 and FU2 at T1 and T2. 3. Calculate $\Delta FU = FU2 - FU1$. 4. Calculate AFC generated: $\Delta FU \div$ slope of standard curve = pmol AFC. 5. Convert to nmol: pmol ÷ 1000. 6. Calculate enzyme activity using the formula: Activity (U/µL) = (nmol AFC × DF) / (ΔT × V) Where: DF = dilution factor of sample (DF=1 if undiluted) ΔT = reaction time (T2-T1) in minutes V = volume of sample added (µL)

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