

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

## Chymotrypsin Assay Kit

Catalog No.: BC00183

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

|                       |                                                                        |
|-----------------------|------------------------------------------------------------------------|
| ✉ Email (Sale)        | <a href="mailto:order@enkilife.com">order@enkilife.com</a>             |
| ✉ Email (Techsupport) | <a href="mailto:techsupport@enkilife.com">techsupport@enkilife.com</a> |
| ☎ Tel:                | 0086-27-87002838                                                       |
| 🌐 Website:            | <a href="http://www.enkilife.com">www.enkilife.com</a>                 |

**Shelf life:** Please refer to the label on the outer package.

**Techsupport:** In order to provide you with better service, please inform us the lot number on the label of the outer package.

## Basic Information

|                             |                                               |
|-----------------------------|-----------------------------------------------|
| <b>Product Name</b>         | Chymotrypsin Assay Kit                        |
| <b>Detection Method</b>     | Colorimetric                                  |
| <b>Sample Type</b>          | Tissue                                        |
| <b>Assay Type</b>           | Quantitative                                  |
| <b>Detection Instrument</b> | Spectrophotometer, Microplate reader (660 nm) |

## Product Introduction

Chymotrypsin, also known as chymotrypsin, is a proteolytic enzyme extracted from bovine or porcine pancreas. It acts as an endopeptidase, specifically cleaving the carboxyl-terminal peptide bonds of tyrosine and phenylalanine in protein polypeptide chains. It selectively hydrolyzes carboxyl-terminal aromatic amino acids (tyrosine, tryptophan, leucine) or large-volume hydrophobic residues with side chains such as methionine.

It can decompose fibrin coagulates at inflammatory sites, promote the dissolution and degradation of blood clots, purulent secretions and necrotic tissues, thereby cleansing wound surfaces, facilitating the regeneration of granulation tissue and accelerating wound healing. Its clinical topical application in ophthalmology and dermatology has been well recognized. It is also applicable to wounds and local inflammation to reduce local secretion and edema. In recent years, with the continuous advancement of clinical pharmacological research, its scope of clinical application has become increasingly extensive.

## Principle

Using casein as the substrate, chymotrypsin hydrolyzes proteins to produce phenolic amino acids. These phenolic amino acids can reduce phenol reagent into a blue substance, and the activity of chymotrypsin can be determined via colorimetry.

## Components

| No.          | Components                               | Size     | Notes                                                                                                                                                                                                                                                                                |
|--------------|------------------------------------------|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reagent 1    | Liquid                                   | 24 mL×1  | Store at 4°C                                                                                                                                                                                                                                                                         |
| Reagent 2    | Liquid                                   | 6 mL×2   | Store at 4°C                                                                                                                                                                                                                                                                         |
| Reagent 3    | Liquid                                   | 100 mL×1 | Store at 4°C                                                                                                                                                                                                                                                                         |
| Reagent 4    | Liquid                                   | 9 mL×2   | Store at 4°C away from light                                                                                                                                                                                                                                                         |
| Reagent 5    | 1 mg/mL Tyrosine Standard Stock Solution | 1 mL×1   | Store at 4°C. Preparation of 50 µg/mL standard working solution: Pipette 50 µL of 1 mg/mL standard stock solution, dilute to 1 mL with standard diluent, or dilute at a volume ratio of 1:19. Mix thoroughly. The prepared solution can be stored at 4 °C for approximately 10 days. |
|              | Standard Diluent                         | 20 mL×1  |                                                                                                                                                                                                                                                                                      |
| Reagent 6    | Homogenate Medium                        | 60 mL×2  | Store at 4°C                                                                                                                                                                                                                                                                         |
| Consumable 1 | Microplate                               | 1 plate  | RT                                                                                                                                                                                                                                                                                   |
| Consumable 2 | Plate Sealer                             | 2 pieces | RT                                                                                                                                                                                                                                                                                   |

## Storage

The kit has a validity period of 3 months.

## Preparation

Visible spectrophotometer and 1 cm optical path cuvettes (or microplate reader (660 nm)), vortex mixer, 37 °C water bath, centrifuge, distilled water, and protein assay reagents (available from our company).

- Sample handling**

Accurately weigh the tissue sample. Add 9 times the volume of Reagent 6 at a mass(g):

volume(mL) ratio of 1:9. Perform tissue homogenization under low temperature (0-4°C) to prepare a 10% tissue homogenate. Centrifuge the homogenate at 2500 rpm for 10 minutes, then collect the supernatant and place it on ice for subsequent detection (valid within the same day).

Conduct a preliminary test with 1–2 samples first (the optimal result of the preliminary test is that the difference between sample OD and control OD ranges from 0.2 to 0.3). Select the appropriate homogenate concentration for batch determination afterwards.)

## Operation process

### 1. Enzymatic Reaction:

|                                                                                                                                                                  | Assay tube                                                | Control tube                                |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------|---------------------------------------------|
| Homogenate Supernatant (mL)                                                                                                                                      | 0.04                                                      | 0.04                                        |
| Pre-warm in a 37°C water bath for 2 minutes.                                                                                                                     |                                                           |                                             |
| Reagent 2 (mL)                                                                                                                                                   | 0.2                                                       | 0.2                                         |
|                                                                                                                                                                  | Mix well and incubate accurately at 37 °C for 10 minutes. | Mix well and add the Reagent 1 immediately. |
| Reagent 1 (mL)                                                                                                                                                   | 0.4                                                       | 0.4                                         |
| Mix thoroughly, incubate at 37 °C for 10 minutes, take out and mix again, centrifuge at 4000 rpm for 10 minutes, and collect the supernatant for color reaction. |                                                           |                                             |

### 2. Color Reaction:

|                                         | Blank tube | Standard tube | Assay tube | Control tube |
|-----------------------------------------|------------|---------------|------------|--------------|
| Standard Diluent (mL)                   | 0.3        |               |            |              |
| 50 µg/mL Standard Working Solution (mL) |            | 0.3           |            |              |

|                                                                                                                                                                                                                                                                                                                                                                                                         |     |     |     |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|-----|-----|-----|
| Supernatant of enzymatic reaction (mL)                                                                                                                                                                                                                                                                                                                                                                  |     |     | 0.3 | 0.3 |
| Reagent 3 (mL)                                                                                                                                                                                                                                                                                                                                                                                          | 1.5 | 1.5 | 1.5 | 1.5 |
| Reagent 4 (mL)                                                                                                                                                                                                                                                                                                                                                                                          | 0.3 | 0.3 | 0.3 | 0.3 |
| <p>Mix thoroughly, incubate in a 37 °C water bath for 20 minutes, then take out. Set the wavelength to 660 nm with a 1 cm optical path, and zero the spectrophotometer with distilled water. Measure the absorbance value (A) of each tube. Alternatively, pipette 200 µL of the reaction solution from each tube into a 96-well plate and read the absorbance at 660 nm using a microplate reader.</p> |     |     |     |     |

Notes: Due to differences among individual samples, a control tube shall be prepared for each sample. Only 1-2 tubes are required for the standard group and blank group respectively. If the OD difference between the assay tube and control tube is too small, prolong the first enzymatic reaction time at 37°C for the assay tube (e.g., 20 min or 30 min), to keep the OD difference above 0.01 as far as possible.

## Calculation

Definition: One enzyme activity unit is defined as the amount of enzyme that decomposes protein to produce 1 µg of amino acid per milligram of tissue protein per minute at 37°C.

Calculation formula:

$$\text{Chymotrypsin Activity(U/mg)} = \frac{A_{\text{Assay}} - A_{\text{Control}}}{A_{\text{Standard}} - A_{\text{Blank}}} \times C_{\text{Standard}} \div T \times N \div C_{\text{pr}}$$

### Annotation:

$C_{\text{Standard}}$ : Standard solution concentration, 50 µg/mL

T: Reaction time, 10 min

N: Dilution factor of enzymatic reaction system, 16

$C_{\text{pr}}$ : Tissue homogenate protein concentration, mg/mL

## **Precautions**

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.