# Human Cathepsin B ELISA Kit

# (Double Antibody Sandwich Method)

**96 Wells**

**Catalogue Number: NE-C2162**

**ELISA Kit for the quantitative Measurement of Human Cathepsin B in Protein Purification Process, and End-Product**

**FOR RESEARCH, DEVELOPMENT AND MANUFACTURING USE ONLY!**
**NOT FOR CLINICAL OR DIAGNOSTIC APPLICATIONS!**
**PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

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## 1. BACKGROUND

Cathepsin B is a lysosomal cysteine protease belonging to the papain family and plays critical roles in a wide range of human physiological and pathological processes. It exhibits maximal catalytic activity under acidic conditions and participates in essential physiological functions including intracellular protein degradation, antigen presentation, as well as the processing of hormones and zymogens. Encoded by the CTSB gene, Cathepsin B is initially synthesized as an inactive precursor (pro-Cathepsin B), which undergoes activation following lysosomal acidification.

Recent studies have demonstrated aberrant expression of Cathepsin B in numerous diseases, with prominent involvement in tumor progression. It facilitates tumor cell invasion, metastasis and angiogenesis, and its upregulation correlates with poor clinical prognosis. Moreover, Cathepsin B is implicated in pathological conditions such as neurodegenerative disorders (e.g., Alzheimer's disease), inflammatory responses and arthritis. Given its pivotal functional roles in disease pathogenesis, Cathepsin B has emerged as a promising therapeutic target and biomarker. Multiple selective inhibitors targeting this protease are currently under research or clinical trial evaluation.

## 2. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the double antibody sandwich Enzyme Linked Immunosorbent Assay. The microtiter plate has been pre-coated with anti-Insulin antibody, standards or samples are then added to the microtiter plate wells and Insulin if present, will bind to the antibody pre-coated wells under specific conditions. Then wash the plate to remove unbound substances, add the HRP-conjugated antibody, and incubate to form a double-antibody sandwich complex with the coated antibody, Insulin, and detection antibody. Then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP, and finally turns to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with total Insulin bound in the initial steps. The color is measured by spectrophotometrically with wavelength of 450nm. The concentration of Insulin in samples is then determined by comparing the O.D. of the samples to the standard curve.

## 3. KITS' ADVANCEMENT

1) High Specificity: Capture antibody and detection antibody are monoclonal antibodies, which

respectively identify different epitopes of the antigen and maximizes the specificity of the reaction. No cross-reactivity among homologous analytes.

2) High Stability: The experiment uses high-quality capture antibodies and antigens, and also applies the broad-spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.

## 4. MATERIALS (Note: Store at 2-8℃)

| | REAGENTS | SPECIFICATION | QUANTITY |
|---|---|---|---|
| 1 | Pre-Coated Microplate (Detachable) | 96 wells | 1 plate (Keep Sealed) |
| 2 | Standard (Stock Solution) -1μg/mL | 10μL | 1 vial |
| 3 | HRP-conjugated Detection antibody (100×) | 150μL | 1 vial |
| 4 | TMB Substrates | 10mL | 1 vial (Avoid Light) |
| 5 | Stop Solution | 10mL | 1 vial |
| 6 | Wash Solution (100×) | 10mL | 1 vial |
| 7 | Diluent Buffer (10×) | 10mL | 1 vial |
| 8 | Plate Sealer | | 4 pieces |
| 9 | Instruction Manual | | 1 |

## 5. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000μL Pipettor
2) Multichannel Pipettor
3) 1L Sterilized Ddeionized Water or Ultrapure Water
4) Sterilized EP tubes
5) Absorbent Paper
6) Microplate Reader
7) High-speed Centrifuge
8) Microplate Washer or Washing Bottles
9) Incubator or Water Bath (Optional)
10) Data Analysis and Graphing Software

## 6. PREPARATION BEFORE ASSAY

Please read the kit instruction manual carefully before use. All reactions are performed at room temperature (20-25°C; the same applies hereinafter).

## 7. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30 minutes before using.
2) The preparation of Wash Solution (1×): Add the contents of 10mL of Wash Solution (100×) into 990mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If crystals have formed in the concentrate, allow it to reach room temperature and mix gently until the crystals are completely dissolved.
3) The preparation of Diluent Buffer (1×): Dilute 10mL of Diluent Buffer (10×) with 90mL of deionized water or ultrapure water to prepare 100mL of Diluent Buffer (1×) . If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The buffer is used as the Diluent Buffer for standards, samples and detection antibodies.

## 8. ASSAY PROCEDURES

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean microplate frame, and start the experiment after the strips returned to room temperature (Note: The microplate frame can be reused). The standard may stick to the tube wall due to transport turbulence. Before using, gently shake it or centrifuge for about 2 seconds.
2) Prepare Standards: When preparing the standards, label 7 tubes, S1-S7 and add a certain volume of Diluent Buffer (1×): S1 (799μL), S2 to S7 (each 250μL).

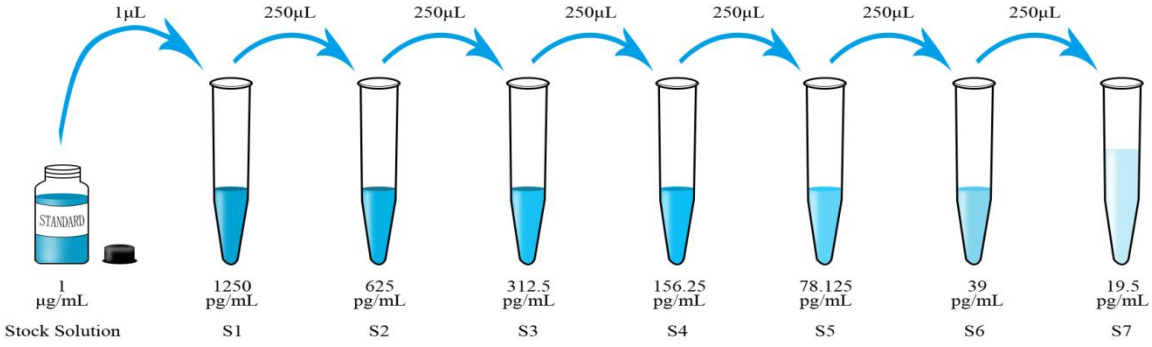
   ① Add 1μL of Standard (Stock Solution - 1μg/mL) into the S1. Mix gently by pipetting up and down twice, then invert to mix thoroughly.

   **(All subsequent mixing steps should be performed in the same manner—do not vortex or use any vigorous mixing method.)**

   ② Transfer 250μL of the resulting S1 (1250pg/mL) to the tube S2, then transfer 250μL of the

resulting S2 solution (625pg/mL) to S3, mix gently. Continue this 2-fold serial dilution sequentially through S7 to achieve a final concentration of 19.5pg/mL.

**NOTE:** There are 7 points of diluted standards, S1: 1250pg/mL, S2: 625pg/mL, S3: 312.5pg/mL, S4: 156.25pg/mL, S5: 78.125pg/mL, S6: 39pg/mL, S7: 19.5pg/mL.




3) Sample addition: Add 100μL of standards and samples to be tested into the microplate wells. For the blank control, add only 100μL of sample diluent.
4) Cover the plate with a plate sealer, put it on the oscillator to mix and incubate for 90 minutes at room temperature.
5) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300μL Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times.

**Note:** There should be no moisture appears in the fifth washing step.

6) Prepare Detection Antibody: Extract 100μL of Detection antibody (100×), and add it into 9.9mL Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100μL of above diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 90 minutes at room temperature.
7) Washing Step: Repeat the same procedure as step 5.
8) Add 100μL TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15 minutes. If the color is light, the reaction time can be extended appropriately, but not more than 30 minutes.
9) Add 50μL of Stop Solution to each well to stop the reaction.
10) Run the microplate reader and conduct measurement at 450nm.
11) Data analysis: Four parameter curve fitting is recommended.

## 9. NOTES

### 1) Sample Preparation

a) After collection, the samples should be aliquoted and stored at -20°C (less than 3 months) or -80°C (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) Certain chemical lysis buffers (e.g., SDS, Triton, etc.) may interfere with the assay and should be used with caution.

d) It is recommended that all standards, controls and samples be run in duplicate.Gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) The process of protein purification is often accompanied by complex buffer solutions. To exclude matrix effects, it is recommended to perform a spike recovery test when using each different buffers for the first time, with an acceptable spike recovery range of 80–120% .

Generally, high salt, low PH, polysaccharide, organic solvents, and detergents can result in lower recovery rates.

The specific operation procedure is as follow: prepare the mixture by combining the diluted standard S1 (1250pg/mL) with the test solution matrix at a 1:1 volume ratio (e.g., 50μL of test solution plus 50μL of diluent either with or without 1250pg/mL standard S1). To calculate the spike recovery rate, subtract the background concentration (measured from the unspiked control) from the spiked sample concentration, then divide the result by the theoretical spiked concentration.

**2) Experimental Operation**

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

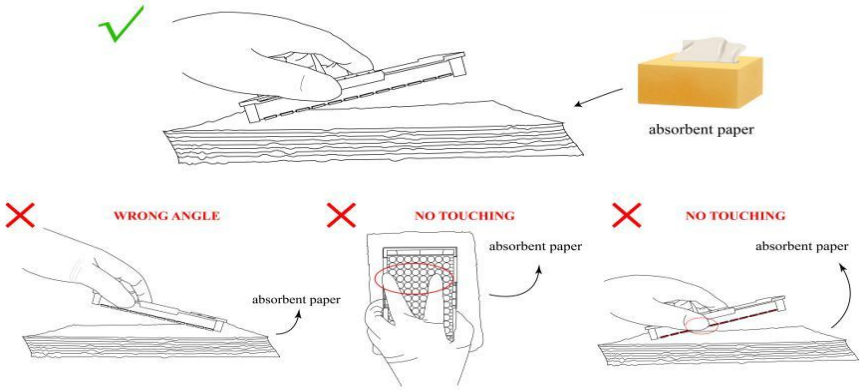
e) TMB is easily contaminated. please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

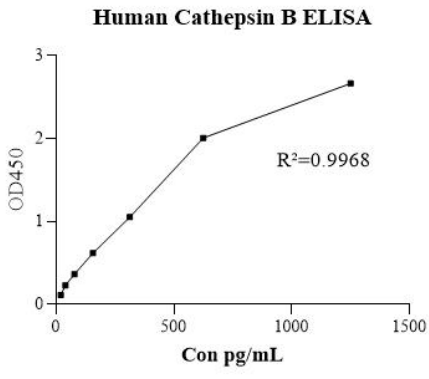
i) Schematic diagram of tapping the plate:




## 10. CALCULATION OF RESULTS

1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.
3) Calculate the concentration of Insulin in the samples corresponding to the mean absorbance from the standard curve.
4) Standard curve for demonstration only.

### APPENDIX 1: EXAMPLE OF STANDARD CURVE




| | Con pg/mL | OD450 |
|---|---|---|
| S1 | 1250 | 2.665 |
| S2 | 625 | 2.010 |
| S3 | 312.5 | 1.055 |
| S4 | 156.25 | 0.619 |
| S5 | 78.125 | 0.366 |
| S6 | 39 | 0.230 |
| S7 | 19.5 | 0.114 |

## 11. QUALITY CONTROL

1) Sensitivity:

LOD: 2.4pg/mL

LOQ: 19.53pg/mL

2) Intra Variation%: <10%

Inter Variation%: <12%

## 12. FAQs & TROUBLESHOOTING

If the experimental results are abnormal, promptly take photos to record the color development results, fully retain the unused strips and reagents, and contact technical support. In addition, you may refer to the troubleshooting information provided below to identify the root cause of the problem.

| Problem Description | Possible Causes | Corresponding Solutions |
|---|---|---|
| Poor Standard Curve | Incorrect dilution of standards | Dilute the standard curve according to the specified ratio. |
| | Inaccurate pipetting or sample loading | Check the pipette and pipette tips. |
| | Incomplete washing of the microplate | Ensure the required number of washing cycles and the volume of washing solution per well. |
| Weak or Absent Color Development | Insufficient incubation time | Ensure adequate incubation time. |
| | Incorrect experimental temperature | Use the recommended incubation temperature. |
| | Insufficient reagent volume or missing addition | Check the pipetting and sample loading process to ensure all reagents are added in sequence with sufficient volume. |
| | Substrate solution not equilibrated to room temperature | Allow the TMB substrate to stand at room temperature for more than 30 minutes before color development. |
| Low OD Value Reading | Incorrect microplate reader settings | Verify the wavelength and filter configuration on the microplate reader.<br>Preheat the microplate reader in advance before reading. |
| High Coefficient of | Incorrect sample loading | Check the sample loading operation. |

| | | |
|---|---|---|
| Variation (CV) | Contamination on the microplate bottom | Inspect the microplate bottom for residual liquid and fingerprints. |
| | Foreign objects or air bubbles in wells | Confirm no foreign objects in wells before sample loading and no air bubbles after loading. |
| | Unsealed or incompletely sealed plate during incubation | Seal the plate with plate sealing film. |
| High Background Value | Incomplete washing of the microplate | Wash the plate according to the method recommended in the instruction manual. |
| | | If using an automated plate washer, check for clogs in all liquid inlets and waste outlets. |
| | | For manual washing, appropriately increase the number of washing cycles. |
| | | Inadequate or missed washing will result in high background. |
| | Incorrect incubation time or temperature | Operate strictly in accordance with the instruction manual. |
| | Contaminated consumables | Ensure the tubes, pipette tips and other consumables are clean. |
| | Contaminated washing solution | Prepare fresh washing solution. |
| | Contaminated substrate solution | The substrate solution is inherently colorless. Ensure the substrate is not contaminated by metal ions or oxidizing agents before use and store it away from light. |
| Low Sensitivity | Improper kit storage | Store all relevant reagents according to the requirements in the instruction manual. |

## 13. CONTACT US

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