NOVATEINBIO
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Human Cathepsin D ELISA Kit

Catalog number: FM-E100178 (96 wells)

The kit is designed to quantitatively detect the levels of Human Cathepsin D in cell culture supernatants, serum, plasma and other suitable sample solution.

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

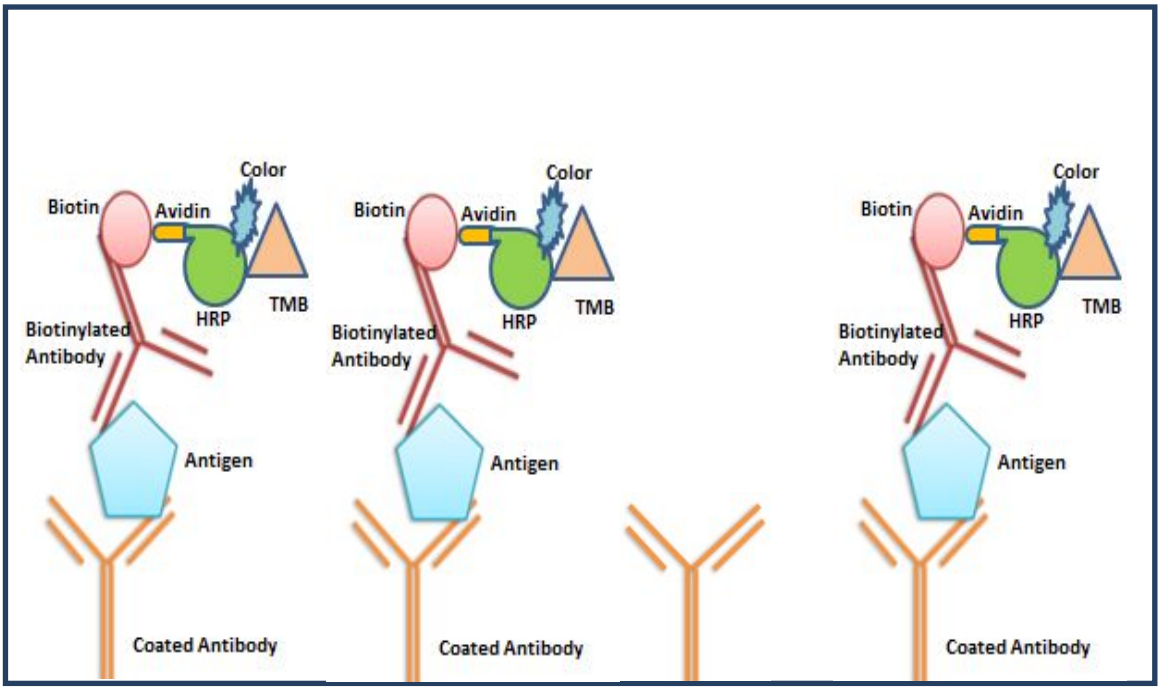
Intended use

The kit is used to quantify the Human Cathepsin D in serum, plasma, body fluids, tissue lysate or cell culture supernatant.

Standard range	156 - 10000 pg/ml
Sensitivity	10.0 pg/ml
Assay time	4 hours
Validity	Six months
Store at	2-8 °C

Assay principle

This Human Cathepsin D ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Human Cathepsin D specific antibody has been precoated onto 96-well plate. The test samples and the biotinylated Human Cathepsin D specific detection antibody are added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP is added and unbound conjugates are washed away with Wash Buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic Stop Solution. The density of yellow is proportional to the Human Cathepsin D amount of sample captured in plate.



Materials supplied

1.Human Cathepsin D standard:	10 ng/vial ×2.
2. 96-well plate pre-coated with anti-Human Cathepsin D Ab:	1.
3. Sample diluent buffer:	12 ml× 2.
4. Detection antibody:	130 µl, dilution 1:100.
5. Streptavidin-HRP:	130 µl, dilution 1:100.
6. Antibody diluent buffer:	12 ml.
7. Streptavidin-HRP diluent buffer:	12 ml.
8. TMB developing agent:	10 ml.
9. Stop Solution:	10 ml.
10. 20 × Wash Buffer:	25 ml.
11. Plate sealer	1.
12. Package insert	1.

Materials required but not supplied

- 37°C incubator.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernatant, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Standard

- Human Cathepsin D: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (10ng /vial) are included in each kit. Use one tube for each experiment.
- 10000pg/ml→156 pg/ml of Human Cathepsin D standard solutions:
- Add 1 ml of sample diluents into one standard tube with 10 ng Human Cathepsin D. Keep the tube at room temperature for 10 minutes and mix thoroughly.This is 10000 pg/ml standard solution.
- Label 7 Eppendorf tubes with 10000pg/ml, 5000 pg/ml,2500 pg/ml,1250 pg/ml,625 pg/ml, 312 pg/ml, 156 pg/ml respectively.Transfer 1.0 ml of 10000 pg/ml standard solution into 10000 pg/ml tube.Then make 2-fold serial dilution from 10000 pg/ml to 156 pg/ml in seven 1.5 ml tubes.
- Make sure each tube has >= 300 ul standard.

  Note: The standard solutions are best used within 2 hours.

Biotinylated anti-Human Cathepsin D antibody working solution

- The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be: 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume.
- Biotinylated anti-Human Cathepsin D detection antibody should be diluted in 1:100 with Antibody diluent buffer and mixed thoroughly.

Preparation of Streptavidin-HRP working solution

- The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1ml/well x the number of wells (Allowing 0.1-0.2 ml more than total volume).
- Streptavidin-HRP should be diluted in 1:100 with Streptavidin-HRP diluent buffer and mixed thoroughly.

Wash Buffer

- If crystals have formed in the 20 × wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20 ×) to a total volume of 500ml with distilled water.

Assay procedures

Bring all reagents to room temperature before use. Human Cathepsin D Standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Human Cathepsin D concentration in samples.

1. Add 100 µl of sample or standards per well. Add 0.1ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate 90 minutes at 37°C. Note: We recommend that each Human Cathepsin D standard solution and each sample is measured in duplicate.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate 60minutes at 37°C.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at 37°C.
6. Repeat the aspiration/wash as in step 2 for five times.
7. Add 90µl of TMB developing agent to each well. Cover and incubate for 20-40 minutes at room temperature (Protect from light. Do not over-develop).
8. Add 90µl Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

Result calculation

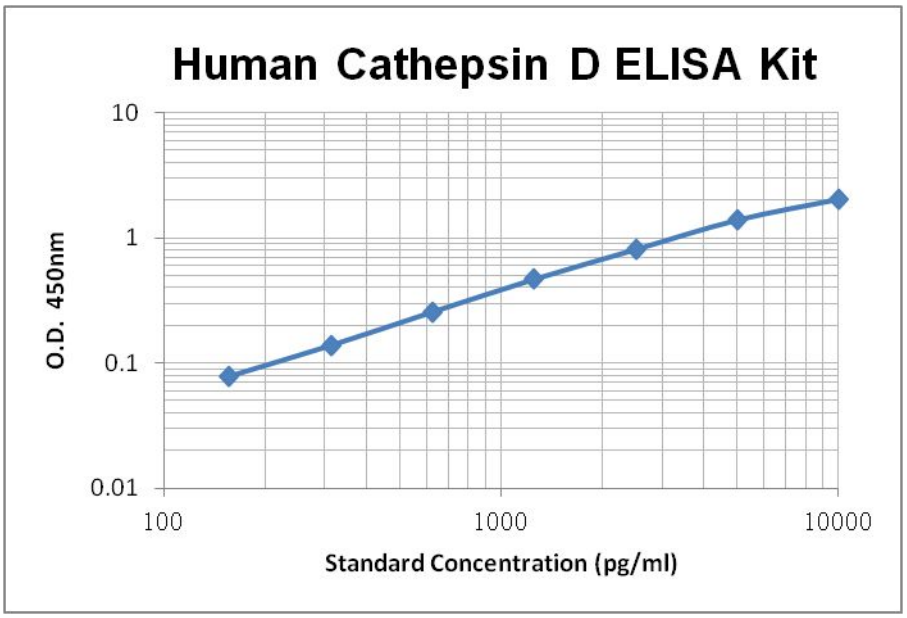
For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Cathepsin D concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical data:

This standard curve was generated at Novatein biolab for demonstration purpose only. A standard curve must be run with each assay.

Conc (pg/ml)	0	156	312	625	1250	2500	5000	10000
O.D.(450nm)	0.022	0.078	0.138	0.255	0.466	0.804	1.382	2.029



Background:

The cathepsins are a group of lysosomal proteases that are active in acidic environments and play an important role in protein degradation. Currently, 11 human cathepsins (B, C, F, H, K, L, O, S, V, W and X) have been shown to have broad substrate specificity. For example, Cathepsins A and G are serine proteases and cathepsins D and E are aspartic proteases. Cathepsins are synthesized as inactive proenzymes and processed to become mature and active enzymes. Human cathepsin D is synthesized as a precursor protein, consisting of a signal peptide, a propeptide, and a mature chain which can further be processed to the light and heavy chains. Cathepsin D is expressed in many different cells and overexpressed in breast cancer cells. It plays a major role in lysosomal protein degradation and also involved in the presentation of antigenic peptides. Deficiency of Cathepsin D in mice showed a progressive atrophy of the intestinal mucosa, a massive destruction of lymphoid organs, and a profound neuronal ceroid lipofucinosis, indicating that cathepsin D is required for regulating cell growth and tissue homeostasis. It is reported that Cathepsin D in human prostate carcinoma cells are responsible for the generation of angiostatin, a potent endogeneous inhibitor of angiogenesis.

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