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ab119584 Cathepsin B Human ELISA Kit

For quantitative detection of Human Cathepsin B in cell culture supernatants, serum and plasma (heparin, EDTA).

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's Human Cathepsin B *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Human Cathepsin B in cell culture supernatants, serum and plasma (heparin, EDTA).

A Cathepsin B specific goat polyclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat, specific for Cathepsin B is then added followed by washing with 1X Wash Buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with 1X Wash Buffer. TMB is then used to visualize the 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 coloration is directly proportional to the Human Cathepsin B amount of sample captured in plate.

Cathepsin B is an enzymatic protein belonging to the peptidase or protease families. In Humans, it is coded by the CTSB gene. And this gene is mapped to chromosome 8p22. The protein encoded by this gene is a lysosomal cysteine proteinase composed of a dimer of disulfide-linked heavy and light chains, both produced from a single protein precursor. It is a member of the peptidase C1 family. Cathepsin B was once suspected as a candidate protease participating in the conversion of β-amyloid precursor protein into the amyloid plaques found in Alzheimer's disease patients. However, this function is now known to be mediated by BACE1 protease. It is now thought that Cathepsin B can degrade β-amyloid precursor protein into harmless fragments. Thus, it is conceivable Cathepsin B may play a pivotal role in the natural defense against Alzheimer's disease. Over expression of Cathepsin B has been associated with esophageal adenocarcinoma and other tumors. The standard product used in this kit is recombinant Human Cathepsin B with the molecular mass of 37KDa.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed - Equilibrate all reagents to room temperature prior to use

Add standard or sample to each well used.

Incubate at room temperature or 37°C.

Add prepared biotin antibody to each well. Incubate at room temperature or 37°C.

Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature or 37°C.

Add TMB to each well. Incubate at room temperature or 37°C. Add Stop Solution to each well. Read the O.D. absorbance.

Incubation can be done at room temperature, but we recommend doing it at 37°C for best consistency with our QC results.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human Cathepsin B antibody Microplate (12 x 8 wells)	96 wells	-20°C
Lyophilized recombinant Human Cathepsin B standard	2 x 10 ng	-20°C
Biotinylated anti-Human Cathepsin B antibody	100 µL	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	100 µL	-20°C
Sample Diluent Buffer	30 mL	-20°C
Antibody Diluent Buffer	12 mL	-20°C
ABC Diluent Buffer	12 mL	-20°C
TMB Color Developing Agent	10 mL	-20°C
TMB Stop Solution	10 mL	-20°C
Plate Seal	4 units	-20°C
Wash Buffer (25X)	20 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader capable of measuring absorbance at 450nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- 100 mL and 1 liter graduated cylinders.
- Eppendorf tubes.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using.

9. Reagent Preparation

- Equilibrate all reagents to room temperature prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Biotinylated anti-Human Cathepsin B

Biotinylated anti-Human Cathepsin B antibody must be diluted in 1:100 with the antibody Diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-Human Cathepsin B antibody to 99 µL antibody Diluent buffer.) The total volume should be: 100 µL /well x (the number of wells). (Allow 100 µL - 200 µL extra for pipetting error).

9.2 1X Avidin-Biotin-Peroxidase Complex

Before use, briefly centrifuge the tubes in case any of the contents are trapped in the lid or sticking to the tube walls. Avidin- Biotin-Peroxidase Complex (ABC) must be diluted in 1:100 with the ABC Diluent Buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume should be: 100 µL/well x (the number of wells). (allow 100 µL - 200 µL extra for pipetting error).

9.3 1X Wash Buffer

Prepare 500 mL of working 1X Wash Buffer by diluting 20 ml of the supplied Wash Buffer (25X) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- Reconstitution of the Human Cathepsin B standard should be prepared no more than 2 hours prior to the experiment. Two tubes of Cathepsin B standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the Cathepsin B standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This 10 ng/mL Standard #1 should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

10.2 Label seven tubes with #2 - 8.

10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.

10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.

10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.

10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #5 through #7.

10.8 **Standard #8** contains no protein and is serves as the zero standard (0pg/ml).

Standard #	Volume to dilute (µL)	Volume of Sample Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Step 10.1			10,000
2	300 µL Standard #1	300	10,000	5,000
3	300 µL Standard #2	300	5,000	2,500
4	300 µL Standard #3	300	2,500	1,250
5	300 µL Standard #4	300	1,250	625
6	300 µL Standard #5	300	625	312
7	300 µL Standard #6	300	312	156
8	N/A	300		0

11. Sample Preparation

11.1 Cell Culture Supernatants:

Remove particulates by centrifugation at 1,500 rpm and 4°C for 10 min, assay immediately or aliquot and store samples at -20°C.

11.2 Serum:

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

11.3 Plasma:

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

11.4 Cell lysate

Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant.

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (100 - 1,000 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL Sample Diluent Buffer
- Medium target protein concentration (10 - 100 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL Sample Diluent Buffer
- Low target protein concentration (156 - 10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL Sample Diluent Buffer
- Very Low target protein concentration (≤ 156 pg/mL). No dilution necessary, or the working dilution is 1:2.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- At least two replicates of each standard, sample, or control is recommended.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Incubation during the assay procedure is recommended at 37°C for best consistency with our QC results. However, they can also be done at room temperature.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections

13.2 Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the zero well.

13.3 Seal the plate with a new plate seal and incubate at 120 minutes at RT (or 90 min. at 37 °C).

13.4 Remove the seal, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

13.5 Add 100 µL of 1X Biotinylated anti-Human Cathepsin B antibody into each well and incubate the plate at 90 minutes at RT (or 60 minutes at 37°C).

13.6 Wash the plate three times with 300 µL 1X Wash Buffer, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
Note: For automated washing, aspirate all wells and wash THREE times with 1X Wash Buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

13.7 Add 100 µL of 1X Avidin-Biotin-Peroxidase Complex working solution into each well and incubate the plate at 40 minutes at RT (or 30 minutes at 37°C).

13.8 Wash plate five times with 1X Wash Buffer, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 13.6 for plate washing method).

13.9 Add 90 µL of prepared TMB color developing agent into each well and incubate plate at 30 minutes at RT (or 15-25 minutes at 37°C).
Note: The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four

most concentrated Human Cathepsin B standard solutions; the other wells show no obvious color.

13.10 Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.

13.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

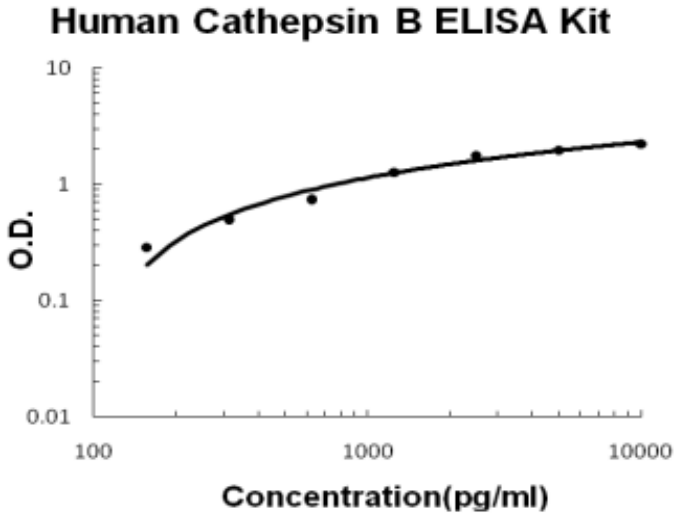
14. Calculations

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 B concentration of the samples can be interpolated from the standard curve.

Δ Note If the samples measured were diluted, make sure to account for this in your calculations.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0	0.070
156	0.284
312	0.498
625	0.739
1,250	1.261
2,500	1.756
5,000	1.969
10,0000	2.216

Figure 1. Example of Cathepsin B standard curve. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

RANGE – 156 - 10,000 pg/mL

SENSITIVITY – < 5 pg/mL

Precision –

Intra-assay precision: (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Sample	Number of measures	Mean (pg/mL)	Standard Deviation	CV%
1	16	252	17.64	7.0
2	16	1,856	129.92	7.0
3	16	4,296	326.49	7.6

Inter-assay precision: (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Number of assays	Mean (pg/mL)	Standard Deviation	CV%
1	24	234	17.85	7.8
2	24	1,923	148.07	7.7
3	24	4,064	377.95	9.3

17. Assay Specificity

This kit detects both endogenous and recombinant Human Cathepsin B.

No detectable cross-reactivity with other relevant proteins.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)