Cathepsin L ELISA

Enzyme immunoassay for the quantitative determination of human cathepsin L in human cell culture supernatants, serum and plasma.

REF BE59211

Σ 96

2-8°C

EU: For research use only.
U.S.: For research use only. Not for use in diagnostic procedures.
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1. INTENDED USE

The Cathepsin L ELISA is an enzyme-linked immunosorbent assay for quantitative detection of Cathepsin L levels in cell culture super-natants, human serum, plasma or other body fluids. The Cathepsin L ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.

2. SUMMARY

Cathepsin L is a lysosomal endopeptidase belonging to the papain cysteine protease superfamily. It is synthesized as pre-proenzyme and after posttranslational modifications and its transport to the lysosomes cathepsin L is participating in the bulk lysosomal protein breakdown or in special cellular functions such as antigen pro-cessing.

Certain specialized cells like macrophages and osteoclasts secrete the precursor of cathepsin L, procathepsin L, which can easily be processed to some form of active enzyme by acid or surface activation or limited proteolysis and which then is involved in connective tissue degradation and the turnover of extracellular matrix proteins.

Like other lysosomal proteinases procathepsin L was also found to be secreted by many malignantly transformed cells and the level of its mRNA and its protein expression and the extent to which it is secreted seem to be correlated to the malignant potential of such cells. Since cathepsin L is capable of degrading protein constituents of the extracellular matrix it is assumed to play a crucial role in tumor progression and metastasis and a number of other disorders where the destruction of the extracellular matrix is the major cause of disease (e.g. rheumatoid arthritis, neurodegeneration).

Inhibition of the enzyme or the proenzyme by low molecular weight inhibitions or antibodies in in vitro and in vivo test systems led to a suppression of the invasive capabilities of malignant cells or a decline in their ability to form tumors.

Cathepsin L is closely related to the recently described cathepsin V (L2) and the sequence indentity of almost 80% between them requires antibodies for the detection of cathepsin L which do not crossreact with
cathepsin V. This requirement can only be met by epitope specific monoclonal antibodies as are used in BE59211.

Elevated levels of cathepsin L have been found in primary cell cultures from cancerous prostatic samples. Higher levels of active cathepsin L were found in breast tumor cells that are invasive, compared to those that are not invasive. Enforced expression of the precursor procathepsin L was shown to confer high tumorigenic and metastatic properties to human melanoma cells. The upregulation of cathepsin L and B by ros Oncogene has been reported for primary human colorectal carcinomas.

Higher levels cathepsin L were found in Rheumatoid arthritis synovial lining compared with osteoarthritis. Cathepsin L activity has been described in aortic aneurysm wall and parietal thrombus acting on proteins determining elasticity and mechanical resistance of arteries.

Cathepsin L was found to be necessary for Li (invariant chain) degradation, a critical step in major histocompatibility complex class II–restricted antigen presentation, in cortical thymic epithelial cells, but not in bone marrow derived antigen–presenting cells.
3. PRINCIPLES OF THE TEST

An anti-Cathepsin L coating antibody is adsorbed onto micro-wells.

Cathepsin L present in the sample or standard binds to anti-bodies adsorbed to the micro-wells; a biotin conjugated anti-Cathepsin L antibody is added and binds to Cathepsin L cap-tured by the first antibody.

Following incubation unbound biotin conjugated anti-Cathepsin L is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-Cathepsin L. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of Cathepsin L present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from five Cathepsin L standard dil-utions and Cathepsin L sample concentration determined.
4. REAGENTS PROVIDED

1 aluminium pouch with a Microwell Plate coated with monoclonal Antibody to human Cathepsin L

1 vial (100 µl) Biotin-Conjugate concentrate anti-Cathepsin L antibody

1 vial (200 µl) Streptavidin-HRP

2 vials Cathepsin L Standard, lyophilized, 100 ng/ml upon reconstitution

1 bottle (50 ml) Wash Buffer Concentrate 20x (PBS with 1% Tween 20)

1 vial (5ml) Assay Buffer Concentrate 20x (PBS with 1% Tween 20 and 10 % BSA)

1 bottle (12ml) Sample Diluent
(Please note: In some cases the Sample Diluent contains insoluble white precipitations which do not interfere with the test performance. Use according to protocol.)

1 vial (15 ml) Substrate Solution

1 vial (12 ml) Stop Solution (1M Phosphoric acid)

1 vial (0.4 ml each) Blue-Dye, Green-Dye, Red-Dye

4 adhesive Plate Covers

Reagent Labels
5. STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage as indicated. Expiry of the kit and reagents is stated on labels.

6. SPECIMEN COLLECTION

Cell culture supernatants, human serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive Cathepsin L. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to 13. E, and F.
7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis.
8. PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

- Do not mix or substitute reagents with those from other lots or other sources.

- Do not use kit reagents beyond expiration date on label.

- Do not expose kit reagents to strong light during storage or incubation.

- Do not pipette by mouth.

- Do not eat or smoke in areas where kit reagents or samples are handled.

- Avoid contact of skin or mucous membranes with kit reagents or specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Avoid contact of substrate solution with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solution must be at room temperature prior to use.

- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.
9. PREPARATION OF REAGENTS

A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Wash Buffer Concentrate (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>25</td>
<td>475</td>
</tr>
<tr>
<td>1 - 12</td>
<td>50</td>
<td>950</td>
</tr>
</tbody>
</table>

B. Assay Buffer

Mix the contents of the bottle well. Add contents of Assay Buffer Concentrate (5.0ml) to 95ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Assay Buffer Concentrate (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>
C. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the Biotin-Conjugate with Assay Buffer (reagent B) in a clean plastic tube as needed according to the following table:

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Biotin-Conjugate (ml)</th>
<th>Assay Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>

D. Preparation of Cathepsin L Standard

Reconstitute Cathepsin L Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Make sure the contents entirely dissolve by gentle swirling. The standard solution obtained is 100 ng/ml.

E. Preparation of Streptavidin-HRP

Make a 1:200 dilution of the concentrated Streptavidin-HRP solution as needed according to the following table:

<table>
<thead>
<tr>
<th>Strips</th>
<th>Streptavidin-HRP (µl)</th>
<th>Assay Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>1 - 12</td>
<td>60</td>
<td>12</td>
</tr>
</tbody>
</table>

F. Addition of colour-giving reagents: Blue-Dye, Green-Dye, Red-Dye

In order to help our customers to avoid any mistakes in pipetting the IBL-International ELISAs, IBL-International now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.
This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (Blue-Dye, Green-Dye, Red-Dye) can be added to the reagents according to the following guidelines:

1. Diluent: Before sample dilution add the **Blue-Dye** at a dilution of 1:250 (see table below) to the diluent (1x) according to the test protocol. After addition of **Blue-Dye**, proceed according to the instruction booklet.

<table>
<thead>
<tr>
<th>Diluent Volume</th>
<th>Dye Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ml</td>
<td>20 µl Blue-Dye</td>
</tr>
<tr>
<td>12 ml</td>
<td>48 µl Blue-Dye</td>
</tr>
</tbody>
</table>

2. Biotin-Conjugate: Before dilution of the concentrated conjugate, add the **Green-Dye** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final conjugate dilution. Proceed after addition of **Green-Dye** according to the instruction booklet, preparation of Biotin-conjugate.

<table>
<thead>
<tr>
<th>Assay Buffer Volume</th>
<th>Dye Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ml</td>
<td>30 µl Green-Dye</td>
</tr>
<tr>
<td>6 ml</td>
<td>60 µl Green-Dye</td>
</tr>
</tbody>
</table>

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP; add the **Red-Dye** at a dilution of 1:250 (see table below) to the Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of **Red-Dye** according to the instruction booklet, preparation of Streptavidin-HRP.

<table>
<thead>
<tr>
<th>Assay Buffer Volume</th>
<th>Dye Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 ml</td>
<td>24 µl Red-Dye</td>
</tr>
<tr>
<td>12 ml</td>
<td>48 µl Red-Dye</td>
</tr>
</tbody>
</table>
10. TEST PROTOCOL

a. Mix all reagents thoroughly without foaming before use.

b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra Microwell Strips coated with Antibody to human Cathepsin L from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. Wash the microwell strips twice with approximately 300 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.

After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µl of Sample Diluent in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (refer to preparation of reagents, 9.D.) Cathepsin L Standard, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure three times, creating two rows of Cathepsin L standard dilutions ranging from 50 ng to 3.1 ng/ml. Discard 100 µl of the contents from the last microwells (E1, E2) used.
Figure 1. Preparation of Cathepsin L standard dilutions:

![Diagram of Cathepsin L standard dilutions]

Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

<table>
<thead>
<tr>
<th>Column</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 1 (50 ng/ml)</td>
<td>Standard 1 (50 ng/ml)</td>
<td>Sample 3</td>
<td>Sample 3</td>
</tr>
<tr>
<td>B</td>
<td>Standard 2 (25 ng/ml)</td>
<td>Standard 2 (25 ng/ml)</td>
<td>Sample 4</td>
<td>Sample 4</td>
</tr>
<tr>
<td>C</td>
<td>Standard 3 (12.5 ng/ml)</td>
<td>Standard 3 (12.5 ng/ml)</td>
<td>Sample 5</td>
<td>Sample 5</td>
</tr>
<tr>
<td>D</td>
<td>Standard 4 (6.25 ng/ml)</td>
<td>Standard 4 (6.25 ng/ml)</td>
<td>Sample 6</td>
<td>Sample 6</td>
</tr>
<tr>
<td>E</td>
<td>Standard 5 (3.12 ng/ml)</td>
<td>Standard 5 (3.12 ng/ml)</td>
<td>Sample 7</td>
<td>Sample 7</td>
</tr>
<tr>
<td>F</td>
<td>Blank</td>
<td>Blank</td>
<td>Sample 8</td>
<td>Sample 8</td>
</tr>
<tr>
<td>G</td>
<td>Sample 1</td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 9</td>
</tr>
<tr>
<td>H</td>
<td>Sample 2</td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 10</td>
</tr>
</tbody>
</table>
e. Add 100 µl of **Sample Diluent** in duplicate to the blank wells.

f. Add 50 µl of **Sample Diluent**, in duplicate, to the sample wells.

g. Add 50 µl of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate** (refer to preparation of reagents).

i. Add 50 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours, if available on a rotator set at 100 rpm.

k. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

l. Prepare **Streptavidin-HRP** (refer to preparation of reagents).

m. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour, if available on a rotator set at 100 rpm.

o. Remove Plate Cover and empty wells. Wash microwell strips 3 times according to point c. of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.
q. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light. The colour development on the plate should be monitored and the substrate reaction stopped (see point r. of this protocol) before positive wells are no longer properly recordable. It is recommended to add the stop solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

r. Stop the enzyme reaction by quickly pipetting 100 µl of Stop Solution into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 – 8°C in the dark.

s. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the Cathepsin L standards.

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.
11. CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the Cathepsin L concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating Cathepsin L for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Cathepsin L concentration.

For samples which have been diluted according to the instructions given in this manual 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).

Note: Calculation of samples with an O.D. exceeding the range of the standard curve may result in incorrect, low Cathepsin L levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual Cathepsin L level.

It is suggested that each testing facility establishes a control sample of known Cathepsin L concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.
Figure 3. Representative standard curve for Cathepsin L ELISA. Cathepsin L was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations. Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.
Typical data using the Cathepsin L ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

<table>
<thead>
<tr>
<th>Standard</th>
<th>Cathepsin L Concentration (ng/ml)</th>
<th>O.D. (450 nm)</th>
<th>O.D. Mean</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>2.046</td>
<td>2.04</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1.182</td>
<td>1.184</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>0.548</td>
<td>0.541</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>0.534</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.25</td>
<td>0.251</td>
<td>0.24</td>
<td>6.5</td>
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<tr>
<td></td>
<td>6.25</td>
<td>0.229</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.12</td>
<td>0.130</td>
<td>0.127</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>3.12</td>
<td>0.123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>0</td>
<td>0.042</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. temperature effects). Furthermore shelf life of the kit may effect enzymatic activity and thus colour intensity. Values measured are still valid.
12. LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
13. PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection of Cathepsin L defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be less than 1.71 ng/ml (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of Cathepsin L. Two standard curves were run on each plate. Data below show the mean Cathepsin L concentration and the coefficient of variation for each sample. The overall intra-assay coefficient of variation has been calculated to be 6.2 %.
<table>
<thead>
<tr>
<th>Positive Sample</th>
<th>Experiment</th>
<th>Cathepsin L Concentration (ng/ml)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>15.8</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.6</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15.6</td>
<td>7.2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>18.8</td>
<td>3.6</td>
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<td>2</td>
<td>17.5</td>
<td>4.3</td>
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<td></td>
<td>3</td>
<td>21.0</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>9.2</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.1</td>
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<td>1</td>
<td>21.0</td>
<td>7.3</td>
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<td>2</td>
<td>20.8</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22.3</td>
<td>5.3</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>26.9</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>23.1</td>
<td>8.8</td>
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<td>3</td>
<td>25.0</td>
<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>7.5</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.0</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6.0</td>
<td>8.8</td>
</tr>
</tbody>
</table>
b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of Cathepsin L. Two standard curves were run on each plate. Data below show the mean Cathepsin L concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 6 %.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cathepsin L Concentration (ng/ml)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.0</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>19.1</td>
<td>9.2</td>
</tr>
<tr>
<td>3</td>
<td>9.2</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>14.0</td>
<td>5.4</td>
</tr>
<tr>
<td>5</td>
<td>21.4</td>
<td>3.6</td>
</tr>
<tr>
<td>6</td>
<td>25.0</td>
<td>7.5</td>
</tr>
<tr>
<td>7</td>
<td>7.1</td>
<td>14.8</td>
</tr>
</tbody>
</table>

C. Spike Recovery

The spike recovery was evaluated by spiking four levels of Cathepsin L into 4 normal human sera. Recoveries were determined in three independent experiments with 4 replicates each. The amount of endogenous Cathepsin L in unspiked serum was subtracted from the spike values. Recoveries ranged from 78 % to 113 % with an overall mean recovery of 92 %.
D. Dilution Parallelism

Four serum samples with different levels of Cathepsin L were assayed at four serial two-fold dilutions with 4 replicates each. In the table below the per cent recovery of expected values is listed. Recoveries ranged from 94% to 118% with an overall mean recovery of 106.2%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Cathepsin L Concentration (ng/ml)</th>
<th>% Recovery of Exp. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expected Value</td>
<td>Observed Value</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>--</td>
<td>51.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25.9</td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>13.3</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6.7</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>--</td>
<td>31.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15.6</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9.1</td>
<td>9.6</td>
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<td>16</td>
<td>4.8</td>
<td>4.8</td>
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<tr>
<td>3</td>
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<td>33.8</td>
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<tr>
<td></td>
<td>4</td>
<td>16.9</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>9.7</td>
<td>10.7</td>
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<tr>
<td></td>
<td>16</td>
<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>--</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>18.2</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10.5</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6.0</td>
<td>5.7</td>
</tr>
</tbody>
</table>
E. Sample Stability

a. Freeze-Thaw Stability
Aliquots of serum and cell culture samples (unspiked or spiked) were stored frozen at -20°C and thawed up to 5 times, and Cathepsin L levels determined. There was no significant loss of Cathepsin L by freezing and thawing up to 5 cycles of freezing and thawing.

b. Storage Stability
Aliquots of a serum and cell culture samples (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the Cathepsin L level determined after 24 h. There was no significant loss of Cathepsin L immunoreactivity during storage.

F. Comparison of Serum and Plasma

From several individuals, serum as well as EDTA, citrate and heparin plasma obtained at the same time point were evaluated. Cathepsin L concentrations were not significantly different and therefore these body fluids are suitable for the assay. It is nevertheless highly recommended to assure the uniformity of blood preparations.

G. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a Cathepsin L positive serum. There was no detectable cross reactivity, notably not with the highly related cathepsin V (80% AS homology).

H. Expected Serum Values

Normal sera from 8 donors were measured. Levels ranged from less than 2.4 ng to 14.2 ng/ml with a mean value of 5 ng/ml.
14. BIBLIOGRAPHY


and immunohistochemistry. Arthritis and Rheumatism 41:1378-1387.


15. ORDERING INFORMATION

For orders please contact:

See last page

For technical information please contact:

e-mail: IBL@IBL-International.com
www.IBL-International.com
16. REAGENT PREPARATION SUMMARY

A. Wash Buffer
Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water.

B. Assay Buffer

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Assay Buffer Concentrate (ml)</th>
<th>Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>2.5</td>
<td>47.5</td>
</tr>
<tr>
<td>1 - 12</td>
<td>5.0</td>
<td>95.0</td>
</tr>
</tbody>
</table>

C. Biotin-Conjugate
Make a 1:100 dilution of Biotin-Conjugate according to the table.

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Biotin-Conjugate (ml)</th>
<th>Assay Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>2.97</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>5.94</td>
</tr>
</tbody>
</table>

D. Standard
Add the volume of distilled water as stated on label to each vial of lyophilized **Cathepsin L Standard** as needed.

E. Streptavidin-HRP

<table>
<thead>
<tr>
<th>Number of Strips</th>
<th>Streptavidin-HRP (ml)</th>
<th>Assay Buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>0.03</td>
<td>6</td>
</tr>
<tr>
<td>1 - 12</td>
<td>0.06</td>
<td>12</td>
</tr>
</tbody>
</table>
17. TEST PROTOCOL SUMMARY

- Wash microwell strips twice with Wash Buffer
- Add 100 µl Sample Diluent, in duplicate, to all standard wells
- Pipette 100 µl reconstituted Cathepsin L Standard into the first wells and create standard dilutions ranging from 50 to 3.12 ng/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells
- Add 100 µl Sample Diluent, in duplicate, to the blank wells
- Add 50 µl Sample Diluent to the sample wells
- Add 50 µl Sample, in duplicate, to designated wells
- Prepare Biotin-Conjugate
- Add 50 µl of diluted Biotin-Conjugate to all wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on microplate shaker
- Prepare Streptavidin-HRP
- Empty and wash microwell strips 3 times with Wash Buffer
- Add 100 µl of diluted Streptavidin-HRP to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C) on microplate shaker
- Empty and wash microwell strips 3 times with Wash Buffer
- Add 100 µl of TMB Substrate Solution to all wells including blank wells
- Incubate the microwell strips for about 10 minutes at room temperature (18° to 25°C)
- Add 100 µl Stop Solution to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low Cathepsin L levels. Such samples require further dilution with Sample Diluent in order to precisely quantitate the actual Cathepsin L level.
Symbols / Symbole / Symbôles / Símbolos / Σύμβολα

<table>
<thead>
<tr>
<th>REF</th>
<th>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td>LYO</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωµα</td>
</tr>
<tr>
<td>IVD</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασµένο</td>
</tr>
<tr>
<td>VD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.</td>
</tr>
<tr>
<td></td>
<td>Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluación. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγηση.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell’uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
<tr>
<td></td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!</td>
</tr>
</tbody>
</table>

Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύµβολα των συστατικών του κιτ συµβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

---

**IBL AFFILIATES WORLDWIDE**

<table>
<thead>
<tr>
<th>IBL International GmbH</th>
<th>Tel.: + 49 (0) 40 532891 -0 Fax: -11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flughafenstr. 52A, 22335 Hamburg, Germany</td>
<td>E-MAIL: <a href="mailto:IBL@IBL-International.com">IBL@IBL-International.com</a> WEB: <a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>IBL International Corp.</th>
<th>Tel.: +1 (416) 645 -1703 Fax: -1704</th>
</tr>
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<tbody>
<tr>
<td>194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada</td>
<td>E-MAIL: <a href="mailto:Sales@IBL-International.com">Sales@IBL-International.com</a> WEB: <a href="http://www.IBL-International.com">http://www.IBL-International.com</a></td>
</tr>
</tbody>
</table>

**LIABILITY:** Complaints will be accepted in each mode –written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2012-01-20