

# Quantikine<sup>®</sup> ELISA

## Human Pro-Cathepsin B Immunoassay

Catalog Number DCATB0

For the quantitative determination of human Pro-Cathepsin B concentrations in cell culture supernates, cell lysates, serum, plasma, saliva, and urine.

**Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.**

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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## INTRODUCTION

The cathepsins are a group of lysosomal proteases that are active in acidic environments and play an important role in protein degradation. Having broad substrate specificity, cathepsins are able to process many molecules, leading to their activation or inactivation. Approximately 11 human cathepsins (B, C, F, H, K, L, O, S, V, W and X) are cysteine proteases of the papain family, sharing similar amino acid (aa) sequences and folds (1). Cathepsins A and G are serine proteases and cathepsins D and E are aspartic proteases (2). Expression and activity of cathepsins are regulated at different levels. Cathepsins are synthesized as inactive proenzymes and processed to become mature and active enzymes. Endogenous protein inhibitors, such as cystatins and some serpins, inhibit active cathepsin enzyme activity.

Human Cathepsin B precursor consists of 339 amino acid (aa) residues including a signal peptide (aa 1-17), a pro- region (aa 18-79) and a mature chain (aa 80-333) (3). The active protease is known to process a number of proteins, including pro- and active caspases, pro-renin, and secretory leukocyte protease inhibitor (SLPI). Therefore, Cathepsin B may be important in activation and inactivation of caspases, activation of renin, and inactivation of SLPI, the key steps in apoptosis, angiotensin production, and progression of emphysema (4-6). Cathepsins B and L likely play a pivotal role in maintenance of the central nervous system since their combined deficiency in mice is lethal at two to four weeks of age and characterized by neuronal loss and brain atrophy (7). Because of its increased and redistributed expression pattern in human and animal tumors, pro- or active Cathepsin B may be a marker for invasion and metastasis of various cancers, such as advanced melanoma, breast cancer, and colorectal cancer (8-11).

The Quantikine Human Pro-Cathepsin B Immunoassay is a 4.5 hour solid phase ELISA designed to measure the pro- form of cathepsin B in cell culture supernates, cell lysates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human Pro-Cathepsin B and antibodies raised against the recombinant factor. Natural human Pro-Cathepsin B showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards, indicating that this kit can be used to determine relative levels of natural human Pro-Cathepsin B.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Cathepsin B has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Pro-Cathepsin B present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Cathepsin B is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human Pro-Cathepsin B bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## PRECAUTIONS

Human Pro-Cathepsin B is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Pro-Cathepsin B Microplate	892536	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human pro-Cathepsin B.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*  May be stored for up to 1 month at 2-8 °C.*
Human Pro-Cathepsin B Conjugate	892537	21 mL of a monoclonal antibody specific for human pro-Cathepsin B conjugated to horseradish peroxidase with preservatives.	
Human Pro-Cathepsin B Standard	892538	Recombinant human pro-Cathepsin B in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	
Assay Diluent RD1-34	895265	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-34	895828	21 mL of a buffered protein base with preservatives. <i>Use undiluted for serum/plasma samples. Use diluted 1:2 for cell culture supernate/cell lysate/saliva/urine samples.</i>	
Cell Lysis Buffer 3	895366	21 mL of a buffered solution with preservatives. <i>Use diluted 1:5 in this assay. May turn yellow over time.</i>	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

\* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- 2-8 °C refrigerator.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human Pro-Cathepsin B Controls (optional; R&D Systems, Catalog # QC58).

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Cell Lysates** - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate and EDTA plasma are not suitable for use in this assay.*

**Saliva** - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Saliva collector must not have any protein binding or filtering capabilities.*

**Urine** - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

### Use polypropylene tubes.

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10  $\mu$ L of sample + 190  $\mu$ L of Calibrator Diluent RD5-34.

Saliva samples require at least a 20-fold dilution. A suggested 20-fold dilution is 10  $\mu$ L of sample + 190  $\mu$ L of Calibrator Diluent RD5-34 (diluted 1:2).\*

Cell lysate and urine samples require at least a 2-fold dilution. A suggested 2-fold dilution is 100  $\mu$ L of sample + 100  $\mu$ L of Calibrator Diluent RD5-34 (diluted 1:2).

## CELL LYSIS PROCEDURE

### Use the following procedure for the preparation of cell lysate samples.

1. Centrifuge cells and remove supernates.
2. Add 1 mL of Cell Lysis Buffer 3 (diluted 1:5)\* per  $1.0 \times 10^6$  cells.
3. Vortex to resuspend the pellet and incubate at 37 °C with gentle agitation for 30 minutes.
4. Centrifuge cells at 500 x g for 15 minutes to remove cellular debris, pour off the cell lysate supernate, and assay immediately or aliquot and store at  $\leq -70$  °C.

\* See Reagent Preparation section.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Note:** *Pro-Cathepsin B is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

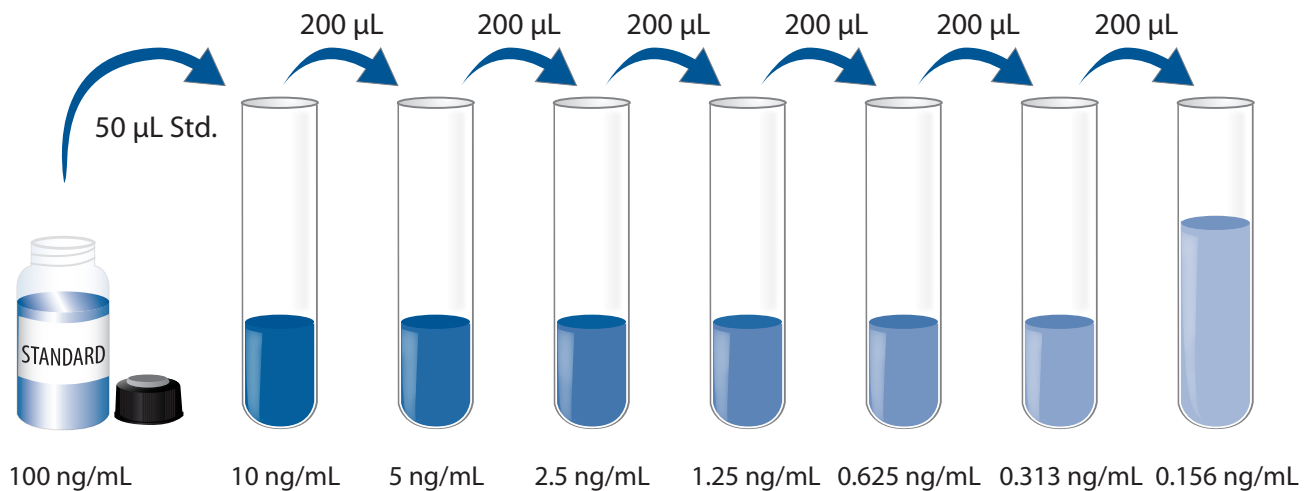
**Calibrator Diluent RD5-34 (diluted 1:2) - For cell culture supernate/saliva/urine/cell lysate samples.** Add 10 mL of Calibrator Diluent RD5-34 to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD5-34 (diluted 1:2).

**Cell Lysis Buffer 3 (diluted 1:5)** - Add 20 mL of Cell Lysis Buffer 3 to 80 mL deionized or distilled water to prepare 100 mL of Cell Lysis Buffer 3 (diluted 1:5).

**Human Pro-Cathepsin B Standard - Refer to the vial label for reconstitution volume.**

Reconstitute the Human Pro-Cathepsin B Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 450  $\mu$ L of Calibrator Diluent RD5-34 (diluted 1:2) (*for cell culture supernate/saliva/urine/cell lysate samples*) or Calibrator Diluent RD5-34 (*for serum/plasma samples*) into the 10 ng/mL tube. Pipette 200  $\mu$ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 ng/mL).





## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.**

**Note:** *Pro-Cathepsin B is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100  $\mu$ L of Assay Diluent RD1-34 to each well.
4. Add 50  $\mu$ L of Standard, control, or sample\* per well. Cover with the adhesive strip provided. Incubate for 2 hours **at 2-8° C**. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400  $\mu$ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200  $\mu$ L of Human Pro-Cathepsin B Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours **at 2-8 °C**.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu$ L of Substrate Solution to each well. Incubate for 30 minutes **at room temperature. Protect from light.**
9. Add 50  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

\*Samples may require dilution/lysis. See Sample Preparation/Cell Lysis Procedure sections.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

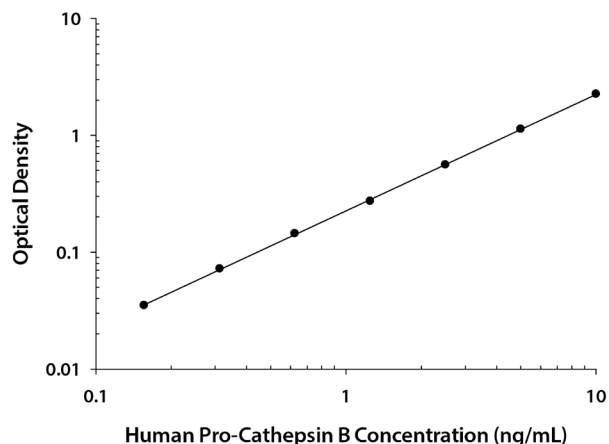
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Pro-Cathepsin B concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

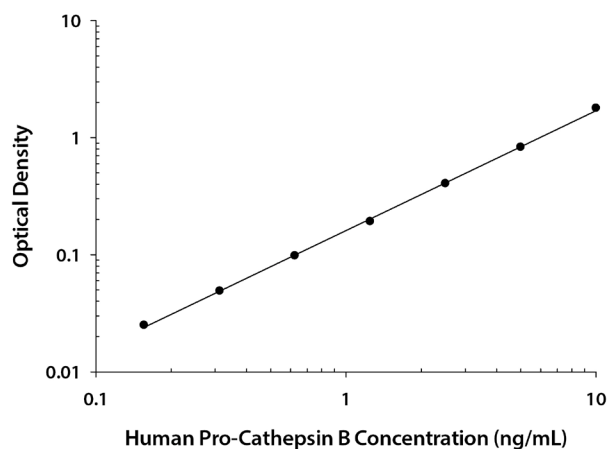
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### CELL CULTURE SUPERNATE/CELL LYSATE/SALIVA/URINE/ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.008 0.008	0.008	—
0.156	0.041 0.044	0.043	0.035
0.313	0.079 0.080	0.080	0.072
0.625	0.150 0.153	0.152	0.144
1.25	0.278 0.283	0.281	0.273
2.5	0.554 0.577	0.566	0.558
5	1.101 1.173	1.137	1.129
10	2.242 2.278	2.260	2.252

### SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.007 0.007	0.007	—
0.156	0.030 0.033	0.032	0.025
0.313	0.054 0.057	0.056	0.049
0.625	0.104 0.105	0.105	0.098
1.25	0.195 0.203	0.199	0.192
2.5	0.409 0.415	0.412	0.405
5	0.833 0.839	0.836	0.829
10	1.783 1.798	1.791	1.784

## PRECISION

### Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

### Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

## CELL CULTURE SUPERNATE/CELL LYSATE/SALIVA/URINE ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	1.08	2.39	4.50	1.16	2.58	4.91
Standard deviation	0.05	0.11	0.23	0.10	0.16	0.30
CV (%)	4.6	4.6	5.1	8.6	6.2	6.1

## SERUM/PLASMA ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	1.32	2.93	5.78	1.48	3.18	5.95
Standard deviation	0.08	0.15	0.29	0.13	0.24	0.42
CV (%)	6.1	5.1	5.0	8.8	7.5	7.1

## RECOVERY

The recovery of human Pro-Cathepsin B spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	98-115%

## LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human Pro-Cathepsin B were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Cell lysates (n=4)	Serum (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	97	100	103	99	102	104
	Range (%)	91-101	98-105	98-106	97-100	98-105	99-109
1:4	Average % of Expected	96	98	104	98	104	107
	Range (%)	87-105	95-102	98-110	96-99	100-108	89-124
1:8	Average % of Expected	95	99	101	98	108	124
	Range (%)	88-98	95-106	96-106	94-101	101-113	124-124
1:16	Average % of Expected	95	107	96	97	109	——
	Range (%)	89-101	103-108	89-108	90-104	102-116	——

## SENSITIVITY

Eighty-eight assays were evaluated and the minimum detectable dose (MDD) of human Pro-Cathepsin B ranged from 0.003-0.079 ng/mL. The mean MDD was 0.016 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Pro-Cathepsin B produced at R&D Systems.

## SAMPLE VALUES

**Serum/Plasma/Saliva/Urine** - Samples from apparently healthy volunteers were evaluated for the presence of human Pro-Cathepsin B in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=60)	60	27-126	21.8
Heparin plasma (n=35)	54	29-132	25.0
Saliva (n=8)	104	38-177	54.4

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Urine (n=13)	1.40	39	ND-2.54

ND=Non-detectable

### Cell Culture Supernates/Cell Lysates:

Human peripheral blood cells ( $1 \times 10^6$  cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 5  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for levels of natural human Pro-Cathepsin B.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	0.47	2.12
Stimulated	1.97	15.8

Aliquots of each cell culture supernate was removed and assayed for human Pro-Cathepsin B. The cells were lysed according to the Cell Lysis Procedure and assayed for human Pro-Cathepsin B.

Cell Lines	Cell Culture Supernates (ng/mL)	Cell Lysates (ng/mL)
SW480 human colorectal adenocarcinoma	5.81	3.18
MG-63 human osteosarcoma	108	6.90
HepG2 human hepatocellular carcinoma	13.2	3.34
MCF-7 human breast cancer	2.99	4.26
COLO 205 human colorectal adenocarcinoma	5.01	12.1
EJ human bladder carcinoma	2.96	4.02

## SPECIFICITY

This assay recognizes natural and recombinant human Pro-Cathepsin B.

The factors listed below were prepared at 200 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human Pro-Cathepsin B standard were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

Cathepsin A  
Cathepsin C/DPPI  
Cathepsin D  
Cathepsin L  
Cathepsin O  
Cathepsin S  
Cathepsin V  
Cathepsin X/Z/P  
Cystatin C  
Cystatin D  
Cystatin SA

### Recombinant mouse:

Cathepsin A  
Cathepsin B  
Cathepsin C/DPPI  
Cathepsin D  
Cathepsin E  
Cathepsin H  
Cathepsin X/Z/P  
Cystatin C

Recombinant human Active Cathepsin B cross-reacts approximately 6% in this assay.

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**PLATE LAYOUT**

Use this plate layout to record standards and samples assayed.

12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
2									
1									
	A	B	C	D	E	F	G	H	

# NOTES

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