Quantikine® ELISA

Mouse CXCL13/BLC/BCA-1 Immunoassay

Catalog Number MCX130

For the quantitative determination of mouse B-Lymphocyte Chemoattractant/B Cell-Attracting Chemokine 1 (BLC/BCA-1) concentrations in cell culture supernates, serum, and plasma.

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

B-Lymphocyte Chemoattractant (BLC), also known as B Cell-Attracting chemokine 1 (BCA-1), or CXCL13, is a constitutively produced 16-18 kDa member of the α-, or CXC family of chemokines (1-3). It is synthesized as a 109 amino acid (aa) precursor that contains a 21 aa signal sequence plus an 88 aa mature segment. The mature segment contains four cysteines with no potential N-linked glycosylation sites (3). In the mature region, mouse BLC/BCA-1 shows only 64% aa similarity and 46% aa identity to human BLC/BCA-1 (3, 4). Among α-chemokines, mature BLC/BCA-1 is only 30% aa identical to mature mouse MIP-2 and KC (3, 5, 6). Like other chemokines, it is assumed that BLC forms non-disulfide linked homodimers. Cells known to express BLC are varied in type and include salivary gland epithelium (7), $CD11b^+CD11c^+$ dendritic cells (8, 9), osteoclasts (10), and peritoneal macrophages (11).

The receptor for mouse BLC/BCA-1 is CXCR5, while human BLC/BCA-1 has been shown to bind to both CXCR5 and CXCR3 (3, 12). Chemotactic responses are noted for CXCR5 expressing cells without an accompanying calcium flux (4).

Functionally, BLC/BCA-1 is a chemotactic agent that plays an important role in B and T cell lymphocyte homing. Naive B cells expressing CXCR5 are proposed to migrate into lymphoid tissue under the influence of BLC/BCA-1 (13, 14). Depending on the degree of antigen exposure, various chemokine receptors on B cells will be subsequently up- and/or down-regulated, directing B cells to appropriate anatomical regions for activation (13, 14). Concomitant to B cell migration, there is a specific subset of $CD4^+$ memory T cells that are also $CXCR5^+$. These cells migrate into lymphoid tissue in response to BLC/BCA-1 and provide help to resident B cells for antibody production via inducible costimulator-ligand interaction (15). BLC also promotes B1 B cell migration into omentum and peritoneum. B1 cells are proposed to secrete "natural" IgM antibodies directed against evolutionarily-conserved bacterial constituents. As such, these antibodies form an immediate first-line of defense against abdominal infection (11, 16). In the fetus, BLC synthesized in the intestine chemoattracts $CD4^+CD3^-$ IL-7 $R\alpha^+$ hematopoietic cells to sites of future Peyer's patch development. Local BLC-CXCR5 and IL-7 R ligand interaction stimulates lymphotoxin-β expression on $CD4^+CD3^-$ hematopoietic cells. This leads to $CD4^+CD3^-$ lymphotoxin-β:stromal LTβR interaction with subsequent Peyer's patch development (17). In a heparin-dependent fashion, BLC/BCA-1 has also been reported to form a heterodimer with FGF basic, resulting in the neutralization of FGF basic activity (18).

The Quantikine® Mouse CXCL13/BLC/BCA-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse BLC/BCA-1 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse BLC/BCA-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse BLC/BCA-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring mouse BLC/BCA-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse BLC/BCA-1 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any BLC/BCA-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse BLC/BCA-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of BLC/BCA-1 bound in the initial step. The sample values are then read off the standard curve.

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 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 and 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.
- Sample addition should be completed within 10 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.

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
Mouse BLC/BCA-1 Microplate	892563	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse BLC/BCA-1.	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.*
Mouse BLC/BCA-1 Standard	892565	2 vials of recombinant mouse BLC/BCA-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse BLC/BCA-1 Control	892566	2 vials of recombinant mouse BLC/BCA-1 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse BLC/BCA-1 Conjugate	892564	12 mL of a polyclonal antibody specific for mouse BLC/BCA-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric 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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

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. Refer to the MSDS on our website prior to use.

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 30 µL of sample + 90 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

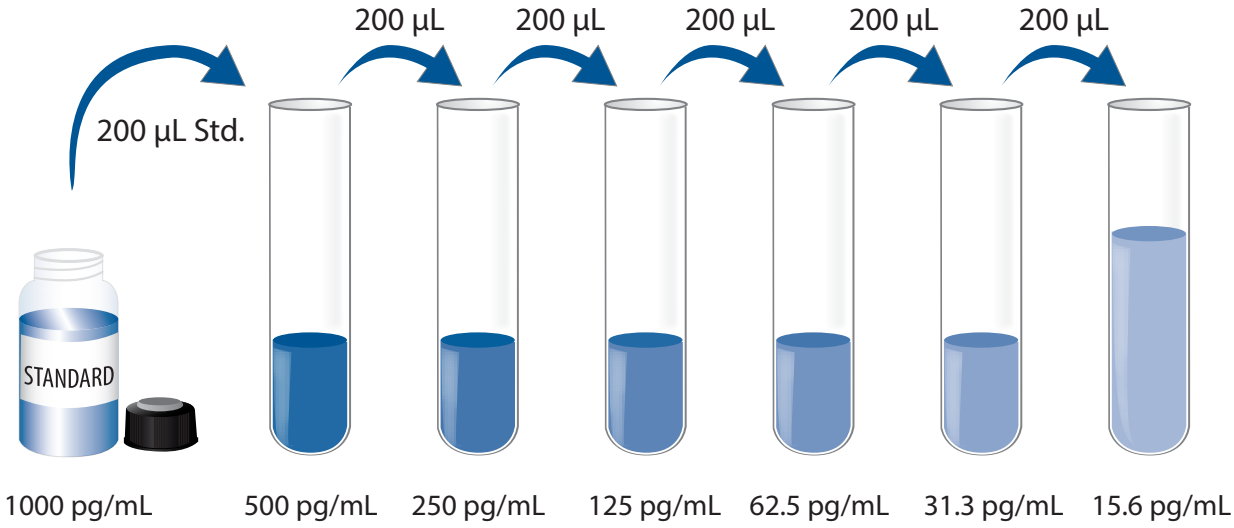
Mouse BLC/BCA-1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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. 100 µL of the resultant mixture is required per well.

Mouse BLC/BCA-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse BLC/BCA-1 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-3 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse BLC/BCA-1 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, standard dilutions, control, 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 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample* per well. Sample addition should be completed within 10 minutes. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) 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 100 µL of Mouse BLC/BCA-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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. See Sample Preparation section.

CALCULATION OF RESULTS

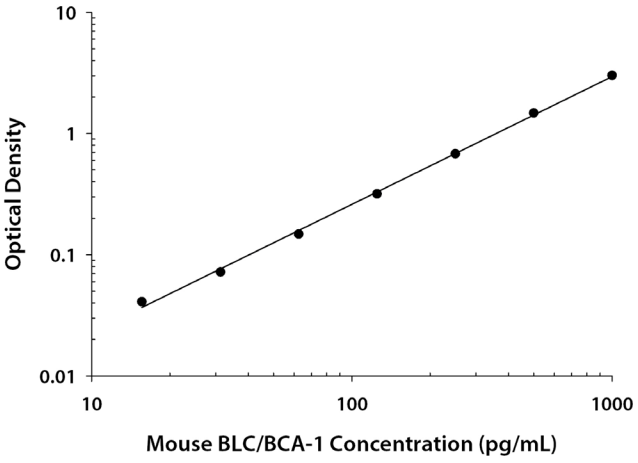
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse BLC/BCA-1 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.030 0.032	0.031	—
15.6	0.072 0.073	0.072	0.041
31.3	0.103 0.103	0.103	0.072
62.5	0.175 0.183	0.179	0.148
125	0.348 0.350	0.349	0.318
250	0.710 0.714	0.712	0.681
500	1.491 1.525	1.508	1.477
1000	3.013 3.081	3.047	3.016

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	51	109	408	52	104	366
Standard deviation	3.1	3.8	15.4	5.1	7.1	27.1
CV (%)	6.1	3.5	3.8	9.8	6.8	7.4

RECOVERY

The recovery of mouse BLC/BCA-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	93	83-109%
Serum* (n=7)	104	85-119%
EDTA plasma* (n=7)	98	80-115%
Heparin plasma* (n=4)	96	80-110%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse BLC/BCA-1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=6)	EDTA plasma* (n=6)	Heparin plasma* (n=6)
1:2	Average % of Expected	105	97	96	96
	Range (%)	93-112	95-99	93-102	89-106
1:4	Average % of Expected	101	89	91	94
	Range (%)	85-111	84-96	87-100	86-106
1:8	Average % of Expected	96	91	94	92
	Range (%)	84-104	82-105	83-112	85-95
1:16	Average % of Expected	101	97	96	93
	Range (%)	88-112	82-119	81-119	86-101

*Samples were diluted prior to assay.

SENSITIVITY

Seventeen assays were evaluated and the minimum detectable dose (MDD) of mouse BLC/BCA-1 ranged from 0.59-2.84 pg/mL. The mean MDD was 1.81 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse BLC/BCA-1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse BLC/BCA-1 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (ng/mL)
Serum	389	75	ND-963
EDTA plasma	224	45	ND-536
Heparin plasma	391	100	67-1369

ND=Non-detectable

Cell Culture Supernates - Two mouse hearts and lungs (1-2 mm pieces in 50 mL of medium) were each cultured for 7 days in RPMI supplemented with 10% fetal bovine serum. Aliquots of the cell culture supernates were removed, assayed for mouse BLC/BCA-1, and measured 212 pg/mL and 262 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse BLC/BCA-1.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the same factors at 50 ng/mL in a mid-range mouse BLC/BCA-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IP-10/CRG-2
I-TAC
LIX
MIG
SDF-1α

Recombinant human:

BLC/BCA-1

Rat serum and cell culture supernate samples were assayed and no cross-reactivity was observed.

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