Quantikine® ELISA

Human IL-10 Immunoassay

Catalog Number D1000B
S1000B
PD1000B

For the quantitative determination of human Interleukin 10 (IL-10) concentrations in cell culture supernates, serum, and plasma.

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

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	5
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
LINEARITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	10

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INTRODUCTION

Interleukin-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1-3). IL-10 is secreted by many activated hematopoietic cell types as well as hepatic stellate cells, keratinocytes, and placental cytotrophoblasts. Whereas human IL-10 is active on mouse cells, mouse IL-10 does not act on human cells (4, 5). Mature human IL-10 shares 86% amino acid sequence identity with equine IL-10 and 72% - 80% with bovine, canine, feline, guinea pig, mouse, ovine, porcine, and rat IL-10. It contains two intrachain disulfide bridges and is expressed as a 36 kDa noncovalently-associated homodimer (4, 6, 7).

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 R*α* is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells (8-13), while the 75 kDa transmembrane IL-10 Rβ is widely expressed (14, 15). The IL-10 dimer binds to two IL-10 R*α* chains, triggering recruitment of two IL-10 Rβ chains (14, 15). IL-10 Rβ does not bind IL-10 directly but is required for signal transduction. IL-10 Rβ also associates with IL-20 R*α*, IL-22 R*α*1, or IL-28 R*α* to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29 (16-18).

The involvement of IL-10 in immunoregulation includes both suppressive and stimulatory effects. It functions as an anti-inflammatory cytokine by inhibiting the expansion and activation of Th1 cells and Th17 cells (19-21) and by promoting the development of M2 macrophages (21). Its expression by immunosuppressive regulatory T cells (Treg) and regulatory B cells is important for Treg proliferation (19). Within a tumor microrenvironment, however, IL-10 inhibits the expansion of Treg as well as myeloid-derived suppressor cells (22, 23). IL-10 induces the intratumoral accumulation and activation of CD8+ T cells (24, 25). IL-10 exerts protective effects including limiting tissue damage in arthritic inflammation (19) and promoting muscle regeneration after injury (21), but it also contributes to the persistence of viral infections (26). The levels of IL-10 are elevated in Sjogren's syndrome (saliva), primary CNS lymphoma (cerebrospinal fluid), and ovarian cancer (serum and ascites) (27-29). Its levels are decreased in the serum in patients with recurrent heart attacks or during preeclampsia and also in the seminal fluid of infertile men (30-32).

The Quantikine Human IL-10 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure IL-10 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human IL-10 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-10 and does not cross-react with viral IL-10. Results obtained using natural human IL-10 showed linear curves that were parallel to the standard curves obtained using this kit standards. These results indicate this kit can be used to determine relative mass values for natural human IL-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IL-10 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 IL-10 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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # D1000B	CATALOG # S1000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-10 Microplate	890227	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-10.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-10 Standard	892646	1 vial	6 vials	Recombinant human IL-10 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human IL-10 Conjugate	892899	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human IL-10 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *For serum/plasma samples.*	
Calibrator Diluent RD5C Concentrate	895046	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *For cell culture supernate samples. Use diluted 1:5 in this assay.*	
Calibrator Diluent RD6P	895118	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

D1000B contains sufficient materials to run an ELISA on one 96 well plate.
S1000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PD1000B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human IL-10 Controls (optional; R&D Systems, Catalog # QC01-1).

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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.

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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

Note: *Hemolyzed samples are not suitable for use this assay.*

SAMPLE PREPARATION

Polypropylene tubes must be used. Do not use glass.

Cell culture supernate samples require at least a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5C (diluted 1:5).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

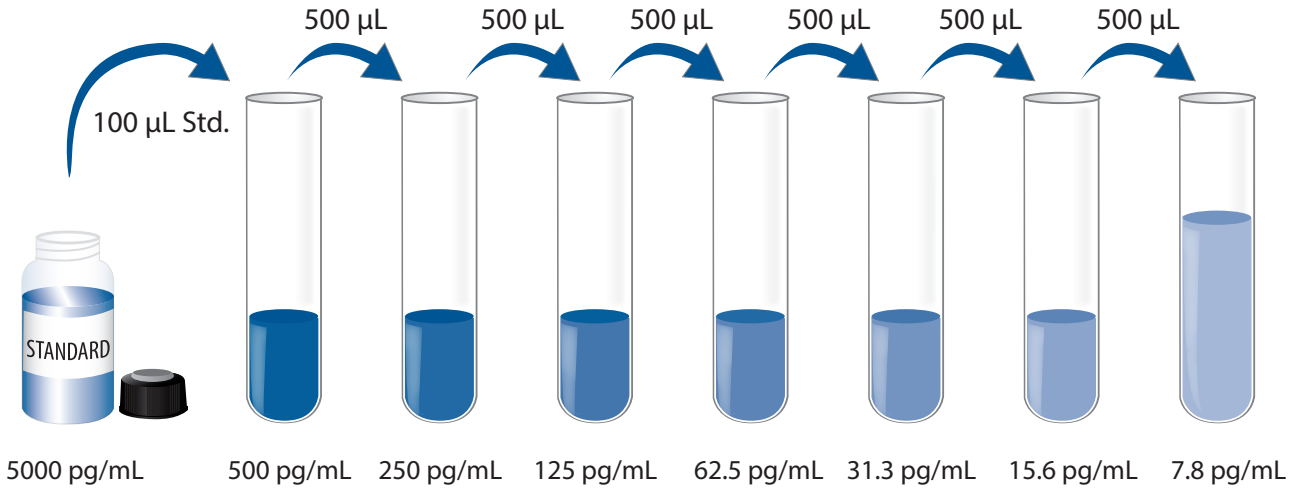
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5C (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5C Concentrate to 80 mL of deionized or distilled water to yield 100 mL of Calibrator Diluent RD5C (diluted 1:5).

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

Human IL-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-10 Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5C (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6P (*for serum/plasma samples*) into the 500 pg/mL tube. Pipette 500 µL 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 500 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 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 standards, samples, and controls be assayed in duplicate.

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. **For Serum/Plasma Samples Only:** Add 50 µL of Assay Diluent RD1W to each well.
4. Add 200 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
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 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 200 µL of Human IL-10 Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1 hour at room temperature.
   For Serum/Plasma Samples: Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. **Protect from light.**
   For Cell Culture Supernate Samples: Incubate for 20 minutes at room temperature.
   For Serum/Plasma Samples: Incubate for 30 minutes at room temperature.
9. Add 50 µL 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. 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.).

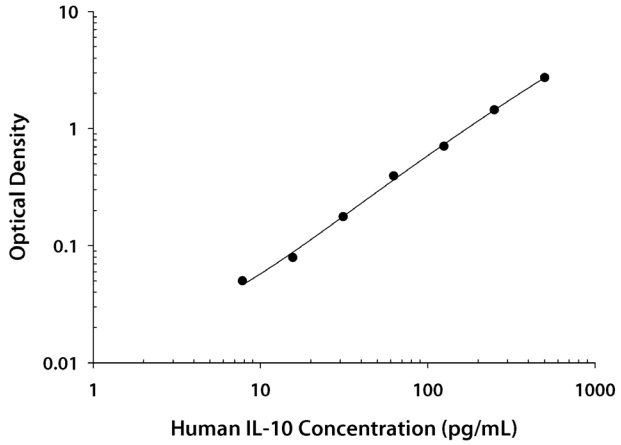
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-10 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

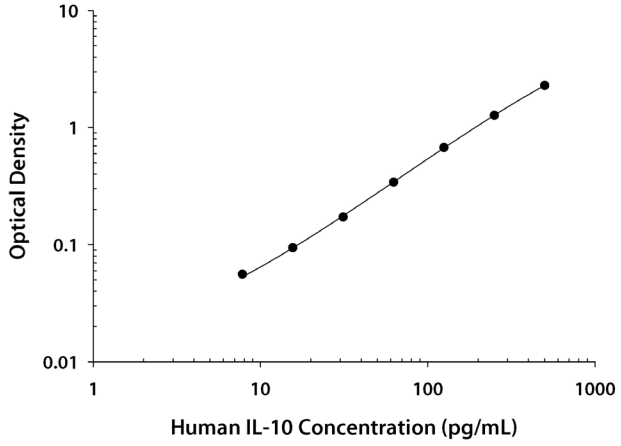
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.032	0.030	—
7.8	0.071 0.088	0.080	0.050
15.6	0.108 0.110	0.109	0.079
31.3	0.203 0.211	0.207	0.177
62.5	0.418 0.432	0.425	0.395
125	0.718 0.755	0.737	0.707
250	1.440 1.506	1.473	1.443
500	2.729 2.779	2.754	2.724

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.040 0.044	0.042	—
7.8	0.091 0.106	0.098	0.056
15.6	0.135 0.137	0.136	0.094
31.3	0.213 0.216	0.214	0.172
62.5	0.378 0.391	0.384	0.342
125	0.705 0.729	0.717	0.675
250	1.305 1.322	1.313	1.271
500	2.330 2.332	2.331	2.289

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 ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	24.4	79.6	223	23.6	74.2	227
Standard deviation	1.6	3.9	5.5	1.8	4.9	12.7
CV (%)	6.6	4.9	2.5	7.6	6.6	5.6

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	23.9	76.9	231	23.2	75.1	228
Standard deviation	1.2	3.3	3.9	1.7	5.6	13.4
CV (%)	5.0	4.3	1.7	7.3	7.5	5.9

RECOVERY

The recovery of human IL-10 spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	98	88-110%
Serum (n=5)	100	92-110%
EDTA plasma (n=5)	92	75-105%
Heparin plasma (n=5)	94	84-105%
Citrate plasma (n=5)	96	87-108%

SENSITIVITY

The minimum detectable dose (MDD) of human IL-10 is typically less than 3.9 pg/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.

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IL-10 in various matrices and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	98	101	100	99	97
	Range (%)	92-108	96-106	97-102	91-104	93-100
1:4	Average % of Expected	96	102	100	100	100
	Range (%)	86-109	97-108	91-106	92-106	93-105
1:8	Average % of Expected	94	102	105	100	97
	Range (%)	84-104	97-109	96-112	93-107	89-104
1:16	Average % of Expected	90	102	108	95	89
	Range (%)	82-98	91-114	99-114	85-110	82-96

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human IL-10 produced at R&D Systems. The NIBSC/WHO Reference Reagent recombinant human IL-10 93/722 was evaluated in this kit.

The dose response curve of the NIBSC/WHO Reference Reagent 93/722 parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human IL-10 kit to approximate NIBSC 93/722 units, use the equation below.

NIBSC (93/722) approximate value (U/mL) = 0.0103 x Quantikine Human IL-10 value (pg/mL)

Note: *Based on data generated in May 2010.*

SAMPLE VALUES

Serum/Plasma - Forty serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human IL-10 in this assay. All samples measured less than the lowest standard, 7.8 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1, 3, and 5 days. Aliquots of the cell culture supernates were removed and assayed for human IL-10.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	98	134	127
Stimulated	2756	3563	2255

SPECIFICITY

This assay recognizes natural and recombinant human IL-10.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:	Other recombinants:	
IL-10 (cytomegalovirus)	IL-10	canine IL-10	porcine IL-10
IL-10 Rα	IL-10 Rα	equine IL-10	rat IL-10
IL-10 Rβ/Fc Chimera		feline IL-10	viral IL-10 (Epstein-Barr)

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