Quantikine™ ELISA

Human IL-23 Immunoassay

Catalog Number D2300B
                S2300B
                PD2300B

For the quantitative determination of human Interleukin 23 (IL-23) concentrations in cell culture supernates, serum, and plasma.

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INTRODUCTION

Interleukin 23 (IL-23) is a heterodimeric cytokine that is related to IL-12 (1-3). It is composed of two disulfide-linked subunits, a p19 subunit that is unique to IL-23, and a p40 subunit that is shared with IL-12 (3-7). The p19 subunit has homology to the p35 subunit of IL-12, as well as to other single chain cytokines such as IL-6 and IL-11. The human p19 subunit cDNA encodes a 189 amino acid (aa) residue precursor protein with a putative 19 aa signal peptide and a 170 aa mature protein. Human and mouse p19 subunits share 70% aa sequence identity. The functional IL-23 receptor complex consists of two receptor subunits, the IL-12 receptor β1 subunit (IL-12 Rβ1) and the IL-23-specific receptor subunit (IL-23 R) (7).

IL-23 is produced by dendritic cells and macrophages in response to pathogens including certain bacteria and viruses and/or their components (3). IL-23 and IL-12 have overlapping but distinct biological activities. The IL-23 immune pathway induces the earliest recruitment of neutrophils to the site of infection while the more classic host defense and cytotoxic response is stimulated by IL-12 (4). IL-12 drives the development of Th1 cells and induces production of IFN-γ by NK cells (3). In contrast, IL-23 has a role in the development/maintenance of a T cell subset characterized by the production of IL-17A, IL-17F, IL-6, and TNF-α (3, 4, 8). The induction of IL-17-producing T cells may involve the actions of TGF-β while their survival and expansion may be IL-23-dependent (9-11). The IL-23/IL-17 axis is an important mediator of inflammation. In mouse models, transgenic over-expression of IL-23 leads to a systemic inflammatory response (12). IL-23 effects on IL-17-producing T cells may also enhance the development of several models of autoimmune disease including experimental allergic encephalomyelitis (EAE), collagen-induced arthritis (CIA), colitis, and diabetes (5, 8, 13-17). IL-23 may also play a role in increased tumor growth associated with chronic inflammation (18). In humans, IL-23 has been found upregulated in several pathologies with dysregulated immune function including psoriasis, Crohn's disease, and multiple sclerosis (19-21).

The Quantikine™ Human IL-23 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human IL-23 in cell culture supernates, serum, and plasma. It contains *Sf*21-expressed recombinant human IL-23 and has been shown to accurately quantitate the recombinant factor. Results obtained for natural human IL-23 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 natural human IL-23.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for the human IL-23 p19 subunit has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-23 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for the human IL-23 p40 subunit 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-23 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, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in 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 # D2300B	CATALOG # S2300B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-23 Microplate	892867	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for the human IL-23 p19 subunit.	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.*
Human IL-23 Conjugate	892868	1 vial	6 vials	21 mL of a polyclonal antibody specific for the human IL-23 p40 subunit conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-23 Standard	892869	1 vial	6 vials	Recombinant human IL-23 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	
Assay Diluent RD1-22	895490	1 vial	6 vials	11 mL of a buffered protein solution with preservatives. *May contain crystals. Mix well to resuspend before use.*	
Calibrator Diluent RD5-16	895302	1 vial	6 vials	21 mL of diluted animal serum with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL 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.

D2300B contains sufficient materials to run an ELISA on one 96 well plate.
S2300B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD2300B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human IL-23 Microplate	892867	50 plates
Human IL-23 Conjugate	892868	50 vials
Human IL-23 Standard	892869	25 vials
Assay Diluent RD1-22	895490	50 vials
Calibrator Diluent RD5-16	895302	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Inserts	751306	2 booklets

*If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com

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
- Human IL-23 Controls (optional; R&D Systems®, Catalog # QC23)

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

Note: *Citrate plasma has not been validated for use in this assay.*
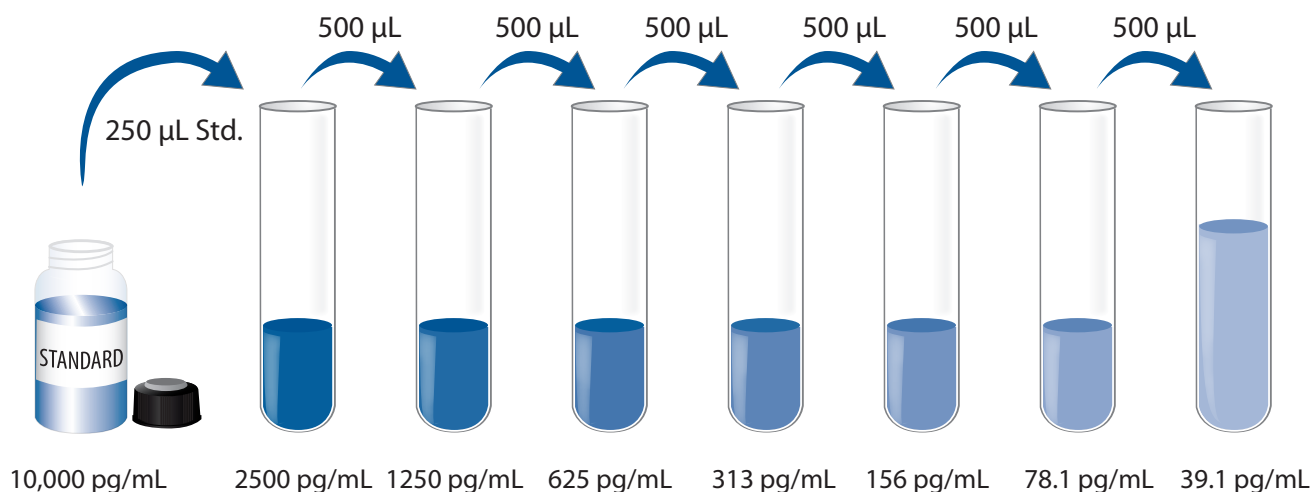
REAGENT PREPARATION

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 480 mL of 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 µL of the resultant mixture is required per well.

Human IL-23 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-23 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 20 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 750 µL of Calibrator Diluent RD5-16 into the 2500 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-16 serves as the zero standard (0 pg/mL).

| 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |

250 µL Std.

STANDARD

| 10,000 pg/mL | 2500 pg/mL | 1250 pg/mL | 625 pg/mL | 313 pg/mL | 156 pg/mL | 78.1 pg/mL | 39.1 pg/mL |

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples 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. Add 100 µL of Assay Diluent RD1-22 to each well. *Assay Diluent RD1-22 may contain crystals. Warm to room temperature and mix well to resuspend before using.*

4. Add 100 µL of standard, control, or sample per well. 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. 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 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-23 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**

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.
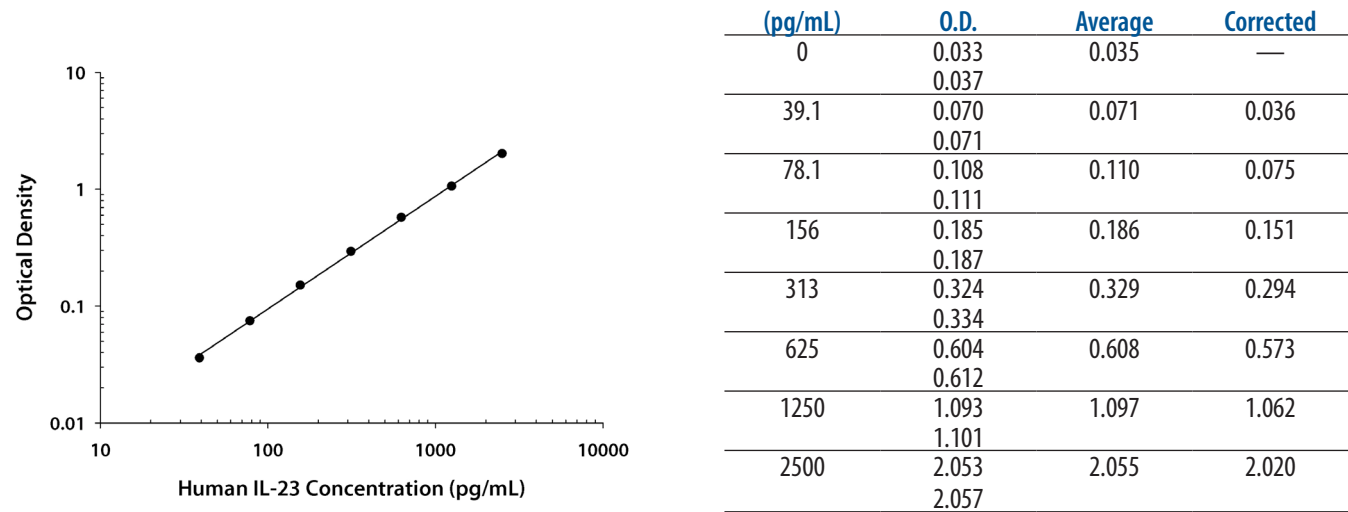
CALCULATION OF RESULTS

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 IL-23 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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	0.033 0.037	0.035	—
39.1	0.070 0.071	0.071	0.036
78.1	0.108 0.111	0.110	0.075
156	0.185 0.187	0.186	0.151
313	0.324 0.334	0.329	0.294
625	0.604 0.612	0.608	0.573
1250	1.093 1.101	1.097	1.062
2500	2.053 2.057	2.055	2.020

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.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	180	496	1170	203	526	1298
Standard deviation	12.0	23.2	49.6	13.3	45.1	78.5
CV (%)	6.7	4.7	4.2	6.6	8.6	6.0

RECOVERY

The recovery of human IL-23 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	93	87-107%
Serum (n=4)	98	88-105%
EDTA plasma (n=4)	95	86-106%
Heparin plasma (n=4)	93	85 - 99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-23 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	100	98	96	97
	Range (%)	96-104	94-105	92-100	94-99
1:4	Average % of Expected	97	100	100	100
	Range (%)	93-104	92-108	97-104	96-103
1:8	Average % of Expected	92	98	98	103
	Range (%)	86-102	92-105	94-101	97-110
1:16	Average % of Expected	91	95	98	104
	Range (%)	——	92-98	96-102	99-113

SENSITIVITY

Forty-three assays were evaluated and the minimum detectable dose (MDD) of human IL-23 ranged from 2.7-16.3 pg/mL. The mean MDD was 6.8 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 *Sf*21-expressed recombinant human IL-23 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Thirty-seven samples from apparently healthy volunteers were evaluated for the presence of human IL-23 in this assay. No medical histories were available for the donors used in this study. One serum sample read 40.5 pg/mL. One EDTA plasma sample read 44.7 pg/mL, and one heparin plasma sample read 44.4 pg/mL.

Cell Culture Supernates:

Fresh peripheral blood mononuclear cells (PBMCs; 1 x 10^6 cells/mL) were purified by positive selection of CD14+ cells and cultured in RPMI supplemented with 10% fetal bovine serum (FBS), 50 μM β-mercaptoethanol, and 25 ng/mL of recombinant human (rh) GM-CSF. After seven days, cells were stimulated with 10 ng/mL of lipopolysaccharide (LPS) for an additional 24 hours. An aliquot of the cell culture supernate was removed, assayed for human IL-23, and measured 113 pg/mL.

Primary human monocytes were cultured in RPMI supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL of streptomycin sulfate, and 25 ng/mL of rhGM-CSF. After seven days, cells were stimulated with 50 ng/mL of LPS for an additional 24 hours. An aliquot of the cell culture supernate was removed, assayed for human IL-23, and measured 2849 pg/mL.

THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% FBS, 50 μM β-mercaptoethanol, and stimulated with 100 ng/mL of LPS for an additional 24 hours. An aliquot of the cell culture supernate was removed, assayed for human IL-23, and measured 587 pg/mL.

THP-1 cells were grown to 60% confluency in RPMI supplemented with 10% FBS, 50 μM β-mercaptoethanol, and stimulated with 1.0 μg/mL of rhIFN-γ for 8 hours followed by 1.0 μg/mL of LPS overnight before the supernate was collected. An aliquot of the cell culture supernate was removed, assayed for human IL-23, and measured 501 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-23.

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 recombinant human IL-23 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Amphiregulin	IL-15
ANG	IL-16
Angiopoietin-2	IL-17
BDNF	IL-19
Cardiotropin-1	IL-20
CD4	IL-22
CD40	IL-23 p19
CD40 Ligand	IL-23 R
CNTF	IL-24
CTLA-4	IL-26 dimer
Epo	IL-26 monomer
Fas	IL-28A
GDNF	IL-29
GITR	Leptin
GITR Ligand	LIF
IFN-γ	MIF
IL-1α	Midkine
IL-1β	NT-3
IL-1ra	NT-4
IL-2	OPG
IL-3	OSM
IL-4	PTN
IL-5	SCF
IL-6	SLPI
IL-7	SMDF
IL-8	TNF-α
IL-9	TNF-β
IL-10	Tpo
IL-11	TRAIL
IL-12	TRANCE
IL-13	

Recombinant mouse:

Cardiotropin-1
CTLA-4
Fas
Fas Ligand
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-11
IL-12
IL-12/IL-23 p40
IL-13
IL-17
IL-23 R
Leptin
LIF
OPG
OPN
OSM
SCF
TNF-α
Tpo
TRANCE

Recombinant rat:

CNTF
GDNF
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
IL-23
Leptin
TNF-α

Recombinant porcine:

IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-8
IL-10
TNF-α

Recombinant mouse IL-23 and recombinant human IL-12/IL-23 p40 cross-react < 0.1% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

NOTES

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