

Quantikine[®] HS ELISA

Human IL-6 Immunoassay

Catalog Number HS600C

SS600C

PHS600C

For the quantitative determination of human Interleukin 6 (IL-6) concentrations in serum, plasma, and urine.

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
PHARMPAK CONTENTS	4
OTHER SUPPLIES REQUIRED	5
PRECAUTIONS.....	5
SAMPLE COLLECTION & STORAGE.....	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
CALCULATION OF RESULTS.....	8
TYPICAL DATA.....	8
PRECISION	9
RECOVERY.....	9
LINEARITY.....	9
SENSITIVITY	10
CALIBRATION	10
SAMPLE VALUES.....	10
SPECIFICITY.....	11
REFERENCES.....	12
PLATE LAYOUT	13

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INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic, α -helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression (1-5). Mature human IL-6 is 183 amino acids (aa) in length and shares 39% aa sequence identity with mouse and rat IL-6 (6). Alternative splicing generates several isoforms with internal deletions, some of which exhibit antagonistic properties (7-10). Cells known to express IL-6 include CD8⁺ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, B1 B cells and pancreatic islet beta cells (2, 11-33). IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids (2). Normal human circulating IL-6 is in the 1 pg/mL range, with slight elevations during the menstrual cycle, modest elevations in certain cancers, and large elevations after surgery (34-38).

IL-6 induces signaling through a cell surface heterodimeric receptor complex composed of a ligand binding subunit (IL-6 R alpha) and a signal transducing subunit (gp130). IL-6 binds to IL-6 R α , triggering IL-6 R α association with gp130 and gp130 dimerization (39). gp130 is also a component of the receptors for CLC, CNTF, CT-1, IL-11, IL-27, LIF, and OSM (40). Soluble forms of IL-6 R α are generated by both alternative splicing and proteolytic cleavage (5). In a mechanism known as trans-signaling, complexes of soluble IL-6 and IL-6 R α elicit responses from gp130-expressing cells that lack cell surface IL-6 R α (5). Trans-signaling enables a wider range of cell types to respond to IL-6, as the expression of gp130 is ubiquitous, while that of IL-6 R α is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (2, 5). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 R α but not from other cytokines that use gp130 as a co-receptor (5, 41).

IL-6, along with TNF- α and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease (1-5). When dysregulated, it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis, and sepsis (2, 5). IL-6 modulates bone resorption and is a major effector of inflammatory joint destruction in rheumatoid arthritis through its promotion of Th17 cell development and activity (1). It contributes to atherosclerotic plaque development and destabilization as well as the development of inflammation-associated carcinogenesis (1, 2). IL-6 can also function as an anti-inflammatory molecule, as in skeletal muscle where it is secreted in response to exercise (2). In addition, it enhances hematopoietic stem cell proliferation and the differentiation of memory B cells and plasma cells (42).

The Quantikine[®] HS Human IL-6 Immunoassay is a 4.0 hour solid-phase ELISA designed to measure human IL-6 in serum, plasma, and urine. It contains *E. coli*-expressed recombinant human IL-6 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-6 showed linear curves that were parallel to the standard curves obtained using the Quantikine[®] HS kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin is added to the wells. After washing away any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 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.
- 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing protein solutions, always avoid foaming.
- 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.
- Assay precision for serum and plasma samples can be further improved with additional centrifugation of the samples. A two minute centrifugation at 13,000 RPM is recommended for serum and plasma samples.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # HS600C	CATALOG # SS600C	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-6 HS Microplate	898933	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-6.	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-6 HS Standard	898935	2 vials	12 vials	Recombinant human IL-6 in a buffered protein base with preservatives; lyophilized. <i>Refer to vial label for reconstitution volume.</i>	Use a fresh standard for each assay. Discard after use.
Human IL-6 HS Conjugate	898934	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-6 conjugated to biotin 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.	
Calibrator Diluent RD5-4	895435	1 vial	6 vials	21 mL/vial of animal serum with preservatives.	
Wash Buffer Concentrate	895003	2 vials	12 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
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).	
Streptavidin Polymer-HRP Diluent	898387	1 vial	6 vials	21 mL/vial of a solution with preservatives.	
Streptavidin Polymer-HRP (100X)	898350	1 vial	6 vials	0.3 mL/vial of Streptavidin Polymer-HRP in a buffer with preservative.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

HS600C contains sufficient materials to run an ELISA on one 96 well plate.

SS600C (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PHS600C). Specific vial counts of each component may vary. 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-6 HS Microplate	898933	50 plates
Human IL-6 HS Standard	898935	25 vials
Human IL-6 HS Conjugate	898934	50 vials
Assay Diluent RD1W	895117	50 vials
Calibrator Diluent RD5-4	895435	50 vials
Wash Buffer Concentrate	895126	12 bottles
Stop Solution	895032	50 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Streptavidin Polymer-HRP Diluent	898387	50 vials
Streptavidin Polymer-HRP (100X)	898350	50 vials
Plate Sealers	N/A	200 sheets
Package Inserts	753296	2 booklets

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
- 1000 mL graduated cylinder
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards
- Human IL-6 HS Controls (optional; R&D Systems®, Catalog # QC246)

PRECAUTIONS

IL-6 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. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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 is not validated for use in this assay.

Grossly hemolyzed samples are not suitable for use in this assay.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of IL-6 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

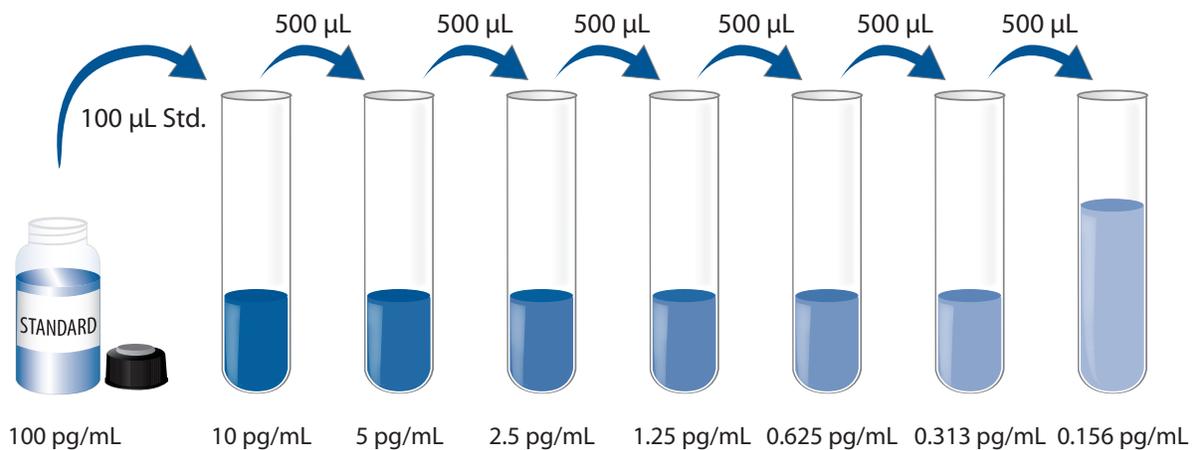
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

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

Streptavidin Polymer-HRP (1X) - Add 0.215 mL of Streptavidin Polymer-HRP (100X) directly to the Streptavidin Polymer-HRP Diluent. Mix well.

Human IL-6 HS Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human IL-6 HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μ L of Calibrator Diluent RD5-4 into the 10 pg/mL tube. Pipette 500 μ L into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 pg/mL standard serves as the high standard. Calibrator Diluent RD5-4 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, controls, and samples be assayed in duplicate.

Note: *High concentrations of IL-6 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents and working standards 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 μ L of Assay Diluent RD1W to each well.
4. Add 100 μ L 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 μ 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 μ L of Human IL-6 HS Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 200 μ L of Streptavidin Polymer-HRP (1X) to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature on the shaker.
9. Repeat the wash as in step 5.
10. Add 200 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
11. Add 50 μ 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.
12. 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

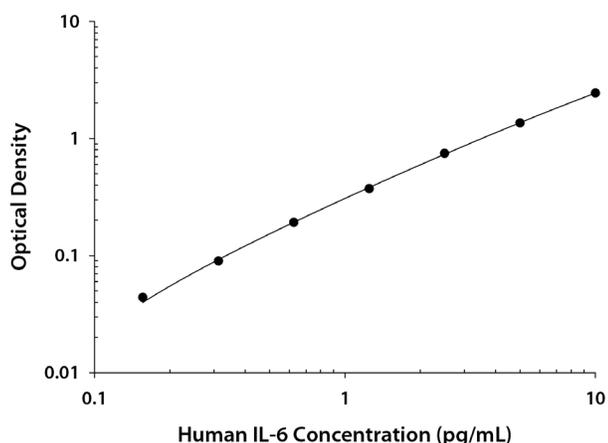
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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-6 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

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	0.051 0.067	0.059	—
0.156	0.101 0.105	0.103	0.044
0.313	0.148 0.149	0.149	0.090
0.625	0.246 0.255	0.251	0.192
1.25	0.431 0.433	0.432	0.373
2.5	0.798 0.809	0.804	0.745
5	1.407 1.429	1.418	1.359
10	2.485 2.510	2.498	2.439

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 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	20	20	20
Mean (pg/mL)	0.527	2.75	5.38	0.528	2.75	5.58
Standard deviation	0.025	0.100	0.223	0.057	0.135	0.220
CV (%)	4.7	3.6	4.1	10.8	4.9	3.9

RECOVERY

The recovery of human IL-6 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Serum (n=4)	95	80-119%
EDTA plasma (n=4)	94	83-108%
Heparin plasma (n=4)	94	83-117%
Urine (n=4)	97	90-103%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-6 were serially diluted with the calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	101	102	99	106
	Range (%)	97-107	95-106	95-104	96-115
1:4	Average % of Expected	104	103	101	108
	Range (%)	102-107	90-108	96-105	97-116
1:8	Average % of Expected	106	108	105	108
	Range (%)	101-113	93-117	101-109	100-117
1:16	Average % of Expected	110	109	112	108
	Range (%)	102-121	89-116	94-125	103-117

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.007-0.090 pg/mL. The mean MDD was 0.031 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 human IL-6 produced at R&D Systems®.

The NIBSC/WHO IL-6 (Human rDNA derived) 1st International Standard 89/548, which was intended as a potency standard, was evaluated in this kit. This standard is a CHO cell-derived recombinant human IL-6. Each ampoule contains a nominal 1.0 µg of glycosylated recombinant human IL-6 and was assigned a unitage of 100,000 International Units/ampoule.

The dose response curve of this 1st International Standard parallels the Quantikine® HS standard curve. To convert sample values obtained with the Quantikine® HS Human IL-6 kit to approximate NIBSC 89/548 mass values, use the equation below.

NIBSC (89/548) approximate value (IU/mL) = 0.087 x Quantikine® HS Human IL-6 value (pg/mL)

Note: Based on data generated in December 2017.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human IL-6 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=30)	1.46	0.495-3.92	0.848
EDTA plasma (n=30)	1.45	0.351-3.48	0.826
Heparin plasma (n=30)	1.51	0.414-3.97	0.876

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=24)	1.04	83	ND-3.48

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-6.

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-6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Cardiotrophin-1	GITR Ligand
CLC	IFN- γ
CLC/CNTF R α	IL-4
CLF-1	IL-6 R
CLF-1/CLC Complex	IL-6 R α /gp130
CNTF	IL-11
CNTF R	IL-11 R α
CNTF R α	IL-31
Epo	Leptin
Fas	Leptin R
gp130	LIF
G-CSF	LIF R α
G-CSF R	Oncostatin M
GDNF	Oncostatin M R β
GITR	

Other recombinants:

canine IL-6
mouse IL-6
rat IL-6

Recombinant porcine IL-6 does not interfere but does cross-react approximately 0.022% in this assay.

Recombinant human IL-6/IL-6 R α Chimera does not interfere but does cross-react approximately 24% in this assay.

REFERENCES

1. Mansell, A. and B.J. Jenkins (2013) Cytokine Growth Factor Rev. **24**:249.
2. Schuett, H. *et al.* (2009) Thromb. Haemost. **102**:215.
3. Erta, M. *et al.* (2012) Int. J. Biol. Sci. **8**:1254.
4. Garbers, C. *et al.* (2012) Cytokine Growth Factor Rev. **23**:85.
5. Mihara, M. *et al.* (2012) Clin. Sci. (Lond.) **122**:143.
6. Hirano, T. *et al.* (1986) Nature **324**:73.
7. Kestler, D.P. *et al.* (1995) Blood **86**:4559.
8. Kestler, D.P. *et al.* (1999) Am. J. Hematol. **61**:169.
9. Bihl, M.P. *et al.* (2002) Am. J. Respir. Cell Mol. Biol. **27**:48.
10. Alberti, L. *et al.* (2005) Cancer Res. **65**:2.
11. May, L.T. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:8957.
12. Sad, S. *et al.* (1995) Immunity **2**:271.
13. Cichy, J. *et al.* (1996) Biochem. Biophys. Res. Commun. **227**:318.
14. Miyazawa, K. *et al.* (1998) Am. J. Pathol. **152**:793.
15. Fried, S.K. *et al.* (1998) Endocrinology **83**:847.
16. Ishimi, Y. *et al.* (1990) J. Immunol. **145**:3297.
17. Jiang, S. *et al.* (1994) Blood **84**:4151.
18. Xin, X. *et al.* (1995) Endocrinology **136**:132.
19. Marz, P. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:3251.
20. Ringheim, G.E. *et al.* (1995) J. Neuroimmunol. **63**:113.
21. Gadiant, R.A. *et al.* (1995) Neurosci. Lett. **194**:17.
22. Kuppner, M.C. *et al.* (1995) Immunology **84**:265.
23. Gagari, E. *et al.* (1997) Blood **89**:2654.
24. Cumberbatch, M. *et al.* (1996) Immunology **87**:513.
25. Fujisawa, H. *et al.* (1997) J. Interferon Cytokine Res. **17**:347.
26. Lee, S.C. *et al.* (1993) J. Immunol. **150**:2659.
27. Lafortune, L. *et al.* (1996) J. Neuropathol. Exp. Neurol. **55**:515.
28. Ericson, S.G. *et al.* (1998) Blood **91**:2099.
29. Melani, C. *et al.* (1993) Blood **81**:2744.
30. Lacy, P. *et al.* (1998) Blood **91**:2508.
31. Jung, H.C. *et al.* (1995) J. Clin. Invest. **95**:55.
32. Spencer, N.F.L. and R.A. Daynes (1997) Int. Immunol. **9**:745.
33. Campbell, I.L. *et al.* (1989) J. Immunol. **143**:1188.
34. D'Auria, L. *et al.* (1997) Eur. Cytokine Netw. **8**:383.
35. Yamamura, M. *et al.* (1998) Br. J. Haematol. **100**:129.
36. Angstwurm, M.W.A. *et al.* (1997) Cytokine **9**:370.
37. Mouawad, R. *et al.* (1996) Clin. Cancer Res. **2**:1405.
38. Sakamoto, K. *et al.* (1994) Cytokine **6**:181.
39. Murakami, M. *et al.* (1993) Science **260**:1808.
40. Muller-Newen, G. (2003) Sci. STKE **2003**:PE40.
41. Mitsuyama, K. *et al.* (2006) Clin. Exp. Immunol. **143**:125.
42. Cerutti, A. *et al.* (1998) J. Immunol. **160**:2145.

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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