Quantikine® HS ELISA

Human IL-6 Immunoassay

Catalog Number HS600B
SS600B
PHS600B

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.

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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 Rα, triggering IL-6 Rα 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 Rα 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 Rα elicit responses from gp130-expressing cells that lack cell surface IL-6 Rα (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 Rα is predominantly restricted to hepatocytes, monocytes, and resting lymphocytes (2, 5). Soluble splice forms of gp130 block trans-signaling from IL-6/IL-6 Rα 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 5.5 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

Due to the high sensitivity of this kit, please note the following precautions.

- **Alkaline phosphatase is detectable in saliva.** Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use and while running the assay (reagent preparation through the addition of Stop Solution).
- Inorganic phosphate is a strong competitive inhibitor of alkaline phosphatase; avoid the use of PBS based wash buffers and other sources of inorganic phosphate contamination.
- Use separate pipettes and glassware for dispensing all reagents to avoid cross-contamination.
- Careful washing of the microplate is essential to minimize non-specific binding of the conjugate.

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, an enzyme-linked polyclonal antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. After an incubation period, an amplifier 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.

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.

Alkaline phosphatase is detectable in saliva. Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use while running the assay (reagent preparation through the addition of Stop Solution).

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.

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

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.
- Although this kit has been designed to eliminate matrix problems, some samples may exist that give falsely elevated values when assayed neat. If serum, plasma, or urine samples generate values that are suspiciously high or higher than the highest standard, dilute the samples in 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

- 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 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.
- Neither the addition of the Substrate Solution nor Stop Solution will result in a color change.
- Addition of the Amplifier Solution will result in a color change (colorless to shades of maroon).
- Amplifier Solution and Stop Solution should be added to the microplate in the same order as 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 # HS600B	CATALOG # SS600B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-6 HS Microplate	892291	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	892293	1 vial	6 vials	Recombinant human IL-6 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-6 HS Conjugate	892292	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-6 conjugated to alkaline phosphatase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-75	895811	1 vial	6 vials	11 mL/vial of a buffered animal serum with preservative. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD6-11 Concentrate	895489	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *Use undiluted for serum/plasma samples. Use diluted 1:2 for urine samples.*	
Wash Buffer Concentrate	895188	1 vial	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Substrate	895884	1 vial	6 vials	Lyophilized NADPH with stabilizers.	Store **in an upright position** for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Substrate Diluent	895885	1 vial	6 vials	7 mL/vial of buffered solution with stabilizers and preservative.	
Amplifier	895886	1 vial	6 vials	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	1 vial	6 vials	7 mL/vial of buffered solution containing INT-violet with stabilizer and preservative.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

HS600B contains sufficient materials to run an ELISA on one 96 well plate.
SS600B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PHS600B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 490 nm, with the correction wavelength set at 650 nm or 690 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 1000 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human IL-6 HS Controls (optional; R&D Systems®, Catalog # QC41).

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 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: *Heparin plasma is not recommended 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: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures (e.g. wear a mask and gloves) to protect reagents during preparation and use while running the assay (reagent preparation through the addition of Stop Solution).*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

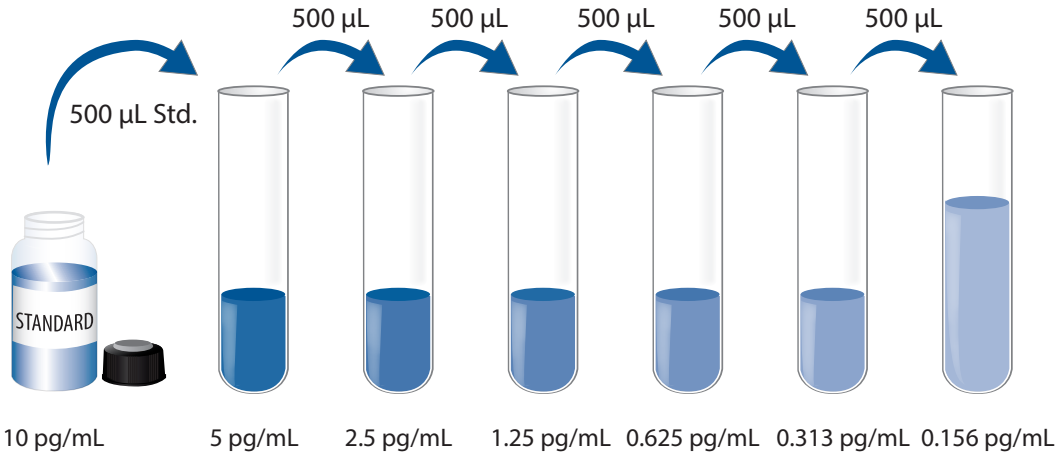
Substrate Solution - Reconstitute the lyophilized Substrate with 6.0 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper** and **re-cap** the vial, and mix thoroughly. Avoid contamination.

Amplifier Solution - Reconstitute the lyophilized Amplifier with 6.0 mL of Amplifier Diluent at least 10 minutes before use. **Re-stopper** and **re-cap** the vial, and mix thoroughly. Avoid contamination.

Calibrator Diluent RD6-11 (diluted 1:2) - **For urine samples only.** Add 10 mL of Calibrator Diluent RD6-11 Concentrate to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-11 (diluted 1:2).

Human IL-6 HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-6 HS Standard with Calibrator Diluent RD6-11 Concentrate (*for serum/plasma samples*) *or* Calibrator Diluent RD6-11 (diluted 1:2) (*for urine samples*). This reconstitution produces a stock solution of 10 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD6-11 Concentrate (*for serum/plasma samples*) or Calibrator Diluent RD6-11 (diluted 1:2) (*for urine samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The reconstituted standard stock serves as the high standard (10 pg/mL). 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 samples and standards be assayed in duplicate.

Note: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures to protect reagents (e.g. wear a mark and gloves).*

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 µL of Assay Diluent RD1-75 to each well. *Assay Diluent RD1-75 may contain a precipitate. Mix well before and during use.*
4. Add 100 µL of standard 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. Wash

   Notes on Washing

   a. Excessive drying of the wells can lead to poor assay performance and imprecision. Subsequent reagents should be added immediately after washing the plate, and the wells should not be allowed to dry. Additionally, avoid prolonged exposure of the wells to vacuum aspiration apparatus.

   b. Inclusion of a 30 second soak between each addition of Wash Buffer and decanting of the plate contents will improve the precision of the assay.

   Wash Procedure

   a. Remove liquid from the wells by inverting the plate and decanting the contents.

   b. Remove excess liquid by grasping the plate firmly and smartly rapping the plate inverted on a clean paper towel at least 5 times.

   c. Fill each well with 400 µL of Wash Buffer using a squirt bottle, manifold dispenser, or autowasher.

   d. Remove liquid from the wells by inverting the plate and decanting the contents or by aspirating the contents with an autowasher.

   e. Repeat steps b-d five times for a total of 6 washes. After the last wash, smartly rap the inverted plate on a clean paper towel at least 10 times to remove excess Wash Buffer.
6. Add 200 µL of Human IL-6 HS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 50 µL of Substrate Solution to each well. Cover with a new adhesive strip. Incubate for 60 minutes at room temperature **on the benchtop. Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature **on the benchtop.**
   Note: *The addition of Amplifier Solution initiates the color development.*
10. Add 50 µL of Stop Solution to each well. The addition of Stop Solution does not affect the color in the wells.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 490 nm. If wavelength correction is available, set to 650 nm or 690 nm. If wavelength correction is not available, subtract readings at 650 nm or 690 nm from the readings at 490 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 490 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.).

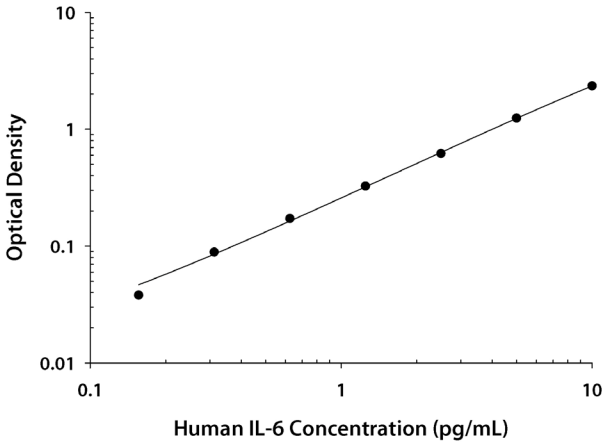
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best 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

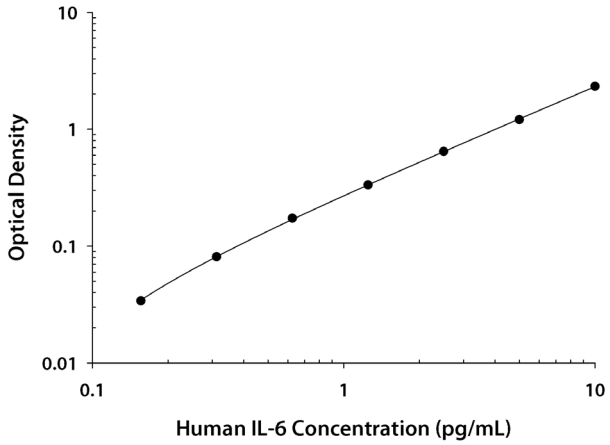
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.130 0.134	0.132	—
0.156	0.166 0.173	0.170	0.038
0.313	0.218 0.223	0.221	0.089
0.625	0.296 0.311	0.304	0.172
1.25	0.447 0.469	0.458	0.326
2.5	0.731 0.773	0.752	0.620
5.0	1.348 1.401	1.375	1.243
10	2.389 2.566	2.478	2.346

URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.133 0.133	0.133	—
0.156	0.165 0.168	0.167	0.034
0.313	0.210 0.217	0.214	0.081
0.625	0.301 0.310	0.306	0.173
1.25	0.445 0.489	0.467	0.334
2.5	0.752 0.806	0.779	0.646
5.0	1.331 1.346	1.339	1.206
10	2.429 2.488	2.459	2.326

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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.

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	36	36	36
Mean (pg/mL)	0.436	2.45	5.53	0.490	2.78	5.65
Standard deviation	0.030	0.19	0.41	0.047	0.20	0.37
CV (%)	6.9	7.8	7.4	9.6	7.2	6.5

URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	35	35	35
Mean (pg/mL)	0.526	3.09	5.77	0.502	2.78	5.62
Standard deviation	0.052	0.18	0.32	0.056	0.19	0.31
CV (%)	9.8	5.8	5.5	11.2	6.8	5.5

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=8)	94	87-99%
EDTA plasma (n=8)	97	84-113%
Citrate plasma (n=8)	106	90-125%
Urine (n=8)	102	93-112%

LINEARITY

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

		Serum (n=8)	EDTA plasma (n=8)	Citrate plasma (n=8)	Urine (n=8)
1:2	Average % of Expected	106	107	102	100
	Range (%)	101-117	99-114	96-112	96-106
1:4	Average % of Expected	107	107	98	98
	Range (%)	94-118	102-115	90-105	91-105
1:8	Average % of Expected	108	103	102	98
	Range (%)	100-120	90-116	93-112	93-102
1:16	Average % of Expected	108	98	108	99
	Range (%)	99-118	78-114	98-120	94-106

SENSITIVITY

Seventy-one assays were evaluated and the minimum detectable dose (MDD) of human IL-6 ranged from 0.016-0.110 pg/mL. The mean MDD was 0.039 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 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 ampule contains a nominal 1 µg of glycosylated recombinant human IL-6 and was assigned a unitage of 100,000 International Units/ampule.

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) = 0.124 x Quantikine® HS Human IL-6 value (pg/mL)

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SAMPLE VALUES

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 of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=52)	1.77	100	0.447-9.96
EDTA plasma (n=35)	1.49	100	0.428-8.87
Citrate plasma (n=16)	1.57	100	0.435-9.57
Urine (n=14)	1.67	93	ND-6.76

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:
Epo
Fas
GDNF
GITR
GITR Ligand
IFN-γ
IL-4

Recombinant mouse:
CTLA-4
Fas
IFN-γ
IL-6

Recombinant rat:
CNTF
GDNF
IFN-γ
IL-1α
IL-1β

Recombinant porcine IL-6 cross-reacts approximately 0.01% in this assay. Recombinant mouse CT-1 cross-reacts approximately 0.004% in this assay.

Recombinant human (rh) gp130 and rhIL-6 R and the rhgp130/rhIL-6 R complex were prepared at physiological levels in calibrator diluent and assayed for cross-reactivity. Preparations of rhgp130, rhIL-6 R, and rhgp130/rhIL-6 R complex in a mid-range recombinant human IL-6 control were assayed for interference. No significant cross-reactivity or interference was observed with gp130. IL-6 R interfered at concentrations ≥ 20 ng/mL. The rhgp130/rhIL-6 R complex interfered at concentrations ≥ 500 ng/mL rhgp130 and ≥ 50 ng/mL rhIL-6 R.

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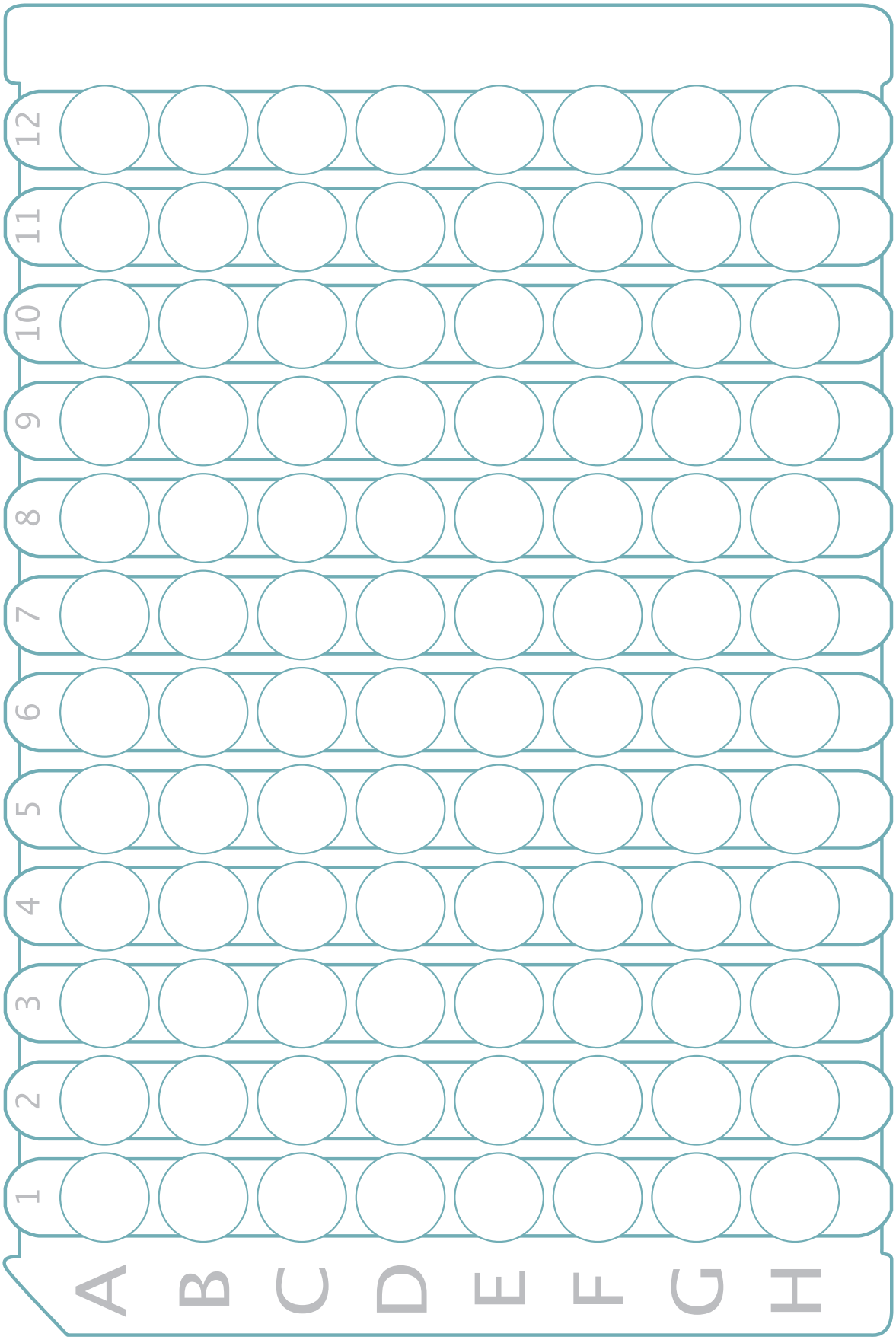
For research use only. Not for use in diagnostic procedures.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

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