Quantikine® HS ELISA

Human IL-7 Immunoassay

Catalog Number HS750

For the quantitative determination of human Interleukin 7 (IL-7) 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.

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INTRODUCTION

Interleukin 7 (previously referred to as lymphopoietin-1 or pre-B-cell growth factor) was originally discovered as a growth factor produced by stromal cells. It is capable of supporting the proliferation of precursor B-lymphocytes ($B220^+$, surface immunoglobulin negative) (1). Mouse IL-7 cDNA was isolated from a stromal cell line by expression cloning and subsequently used as a probe for the cloning of human IL-7 cDNA from a liver adenocarcinoma cell line (2, 3). In addition to being produced by bone marrow stromal cells, IL-7 mRNA has also been detected in the spleen, thymus, and kidney, as well as in keratinocytes (4, 5). Both mouse and human IL-7 have been shown to have pleiotropic effects on a variety of cell types, including cells of the B-, T-, NK-, and myeloid lineages. The biology of IL-7 has been reviewed (4).

The cDNAs for human and mouse IL-7 encode precursor proteins with a 25 amino acid (aa) residue signal peptide that is cleaved to form mature proteins containing essential disulfide bonds and multiple potential sites for N-linked glycosylation. The mature forms of human and mouse IL-7 contain 152 and 129 aa residues, respectively. The greater size of human IL-7 is due, in part, to a contiguous 19 aa residue stretch that is absent in mouse IL-7. At the aa sequence level, there is approximately 60% identity between human and mouse IL-7. Human IL-7 exhibits no species-specificity and is equally active on both human and mouse cells. Although mouse IL-7 is active on human T cells, it was reported to be inactive on human pre-B cells (4, 6).

The biological effects of IL-7 are initiated by binding of the cytokine to a receptor complex consisting of a ligand specific binding component, IL-7 Rα (7), and a second component, the γ_c (gamma common) chain, that is also a part of the receptor complexes associated with IL-2, IL-4, IL-9, and IL-15 binding and signal transduction (8-10). Both components belong to the cytokine receptor superfamily by virtue of sequence homology and by containing the W-S-X-W-S domain, although IL-7 Rα contains only two of the four characteristic conserved cysteines of this family (7, 11, 12). The human IL-7 Rα is produced as a 459 aa residue precursor with a 20 aa residue signal peptide, a 25 aa residue transmembrane domain, an extracellular domain of 219 aa residues, and a cytoplasmic domain of 195 aa residues (7, 12). The cDNA for the mature protein predicts a size of 49.5 kDa, but the observed size of the receptor is approximately 75 kDa, presumably as a result of post-translational glycosylation (7, 12). The mouse IL-7 Rα is the same size and shows approximately 64% sequence identity with the human protein at the aa sequence level (12). Signal transduction following binding of IL-7 to this receptor complex involves association with JAK1 and JAK3 (13), followed by subsequent induction of members of the STAT family, reportedly STAT1, STAT5, and possibly STAT3 (14). Signal transduction also has been reported to involve various members of src-like kinases (15-17), as well as activation of IRS-1 (insulin receptor substrate-1) (18) and PI-3-kinase (phosphatidylinositol-3-kinase) (19). The distinct effects of IL-7 on differentiation of B lymphocytes and proliferation have been shown to be mediated by separate domains of the IL-7 Rα (20).

IL-7 is a pleiotropic cytokine with multiple bioactivities on a variety of cell types (4). Among B-lineage cells, pro-B and pre-B cells, but not mature B cells, can proliferate in response to IL-7 alone. IL-7 may play an important role in T cell development in the thymus. IL-7 has been reported to induce proliferation of immature and mature human and mouse thymocytes. IL-7 was also shown to promote the generation of phenotypically mature $CD45RA^+$ human thymocytes *in vitro* (21), and to induce the V(D)J rearrangement of the T cell receptor β gene in mouse fetal thymocytes (22). In the presence of co-mitogens such as Con A or PHA, IL-7 can stimulate the proliferation of peripheral blood T cells. IL-7 can support the differentiation of cytotoxic T lymphocytes and promote the generation of lymphokine-activated killer cells. Among myeloid lineage cells, IL-7 can up-regulate the production of pro-inflammatory cytokines and stimulate the tumoricidal activity of monocytes/macrophages (4).

The Quantikine® HS Human IL-7 Immunoassay is an 18-24 hour (with an overnight incubation step) solid phase ELISA designed to measure human IL-7 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-7 and antibodies raised against the recombinant factor. It has been shown to quantitate the recombinant factor accurately. Results obtained using natural IL-7 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-7.

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 nonspecific binding of the conjugate.

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-7 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-7 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-7 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-7 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.
- Standards, controls, and samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate 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

- 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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Long incubations are known to cause edge effects in immunoassays. Incubating in a moist environment has been shown to minimize these effects.
- Neither the addition of the Substrate Solution nor the 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 plate 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-7 HS Microplate	890193	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-7.	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-7 HS Conjugate	890194	21 mL of a polyclonal antibody specific for human IL-7, conjugated to alkaline phosphatase with preservatives.	Store for up to 1 month at 2-8 °C.*
Human IL-7 HS Standard	890195	Recombinant human IL-7 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1C	895007	6 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5R	895190	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-44	895376	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer 10X Concentrate	895188	100 mL of a 10-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Substrate	895884	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	7 mL of buffered solution with stabilizers and preservative.	
Amplifier	895886	Lyophilized amplifier enzymes with stabilizers.	
Amplifier Diluent	895887	7 mL of buffered solution containing INT-violet with stabilizer and preservative.	
Plate Sealers	N/A	Adhesive strips.	

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

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 autowasher.
- 1000 mL graduated cylinder.
- Test tubes for dilution of standards.
- Human IL-7 Controls (optional; R&D Systems®, Catalog # QC41).

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 and 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 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 chilled serum collection tube **(Serum separator tubes (SST) are not recommended)** and allow samples to clot **on ice** overnight 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.*

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 and 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 10X Concentrate to 900 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

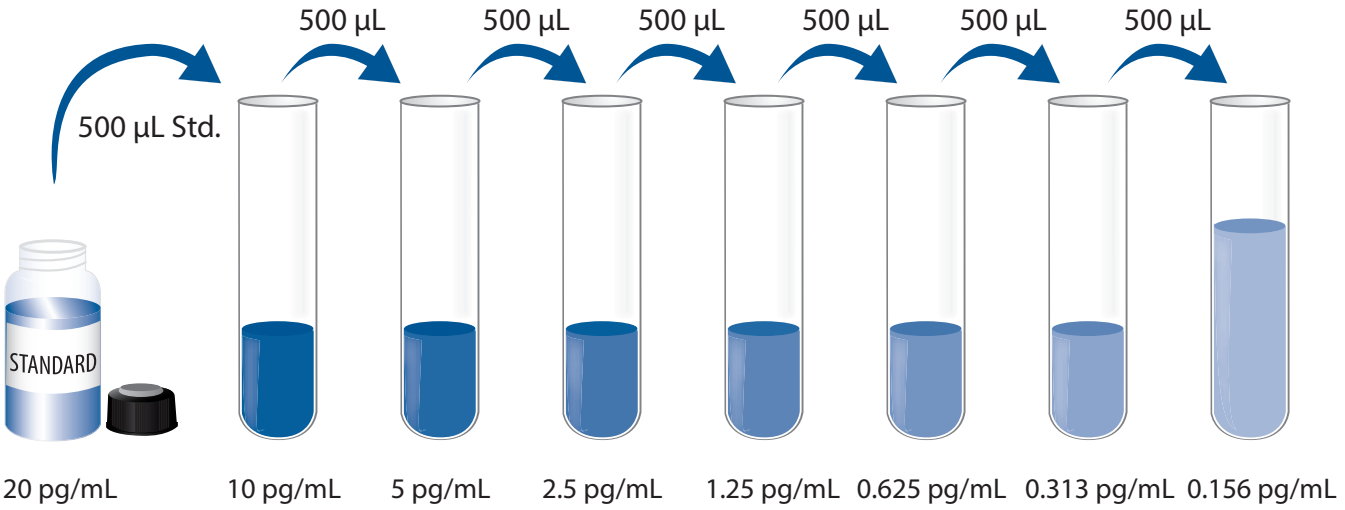
Substrate Solution - Reconstitute the lyophilized Substrate in 6.0 mL of Substrate Diluent at least 10 minutes before use. **Re-stopper and re-cap the vial**, and mix thoroughly. Avoid contamination.

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

REAGENT PREPARATION *CONTINUED*

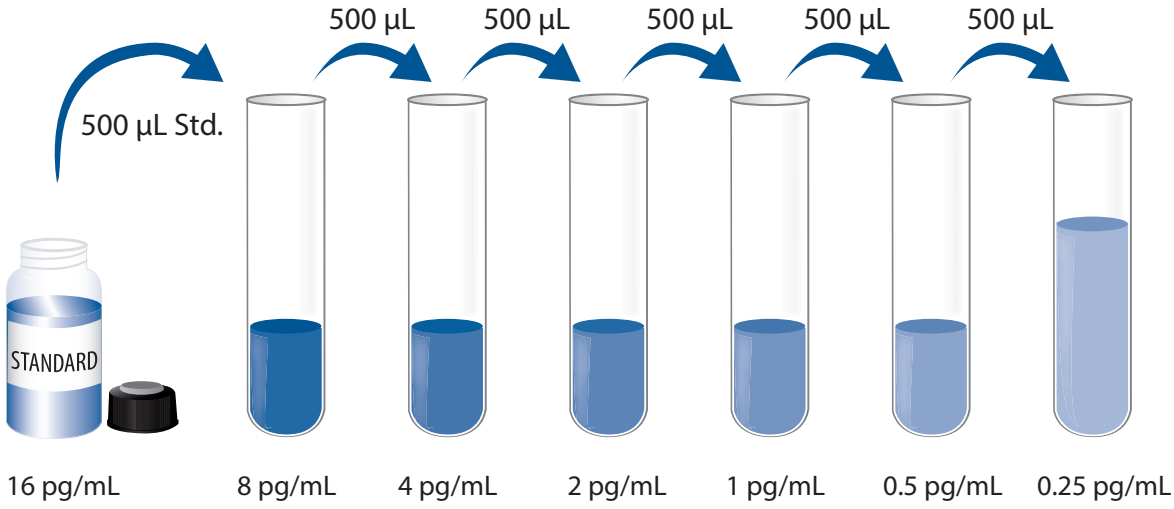
Human IL-7 HS Standard (*Cell Culture Supernate samples*) - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-7 HS Standard with Calibrator Diluent RD5R. This reconstitution produces a stock solution of 20 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5R into each tube. 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 RD5R serves as the zero standard (0 pg/mL).



Human IL-7 HS Standard (*Serum/Plasma samples*) - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-7 HS Standard with Calibrator Diluent RD6-44. This reconstitution produces a stock solution of 16 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 RD6-44 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IL-7 HS Standard (16 pg/mL) serves as the high standard. Calibrator Diluent RD6-44 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: ***Alkaline phosphatase is detectable in saliva.*** *Take precautionary measures to protect reagents (e.g. wear a mask and gloves).*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove any excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1C to each well. *Assay Diluent RD1C may contain a precipitate. Mix well before and during use.*
4. Add 200 µL of standard, control, and sample per well. Cover with the adhesive strip provided. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Incubate for 14-20 hours at room temperature. **Note:** *Long incubations are known to cause edge effects in immunoassays. Incubating in a moist environment has been shown to minimize these effects.* A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process five times for a total of six 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-7 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
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 45 minutes at room temperature. **Do not wash the plate.**
9. Add 50 µL of Amplifier Solution to each well. Cover with a new adhesive strip. Incubate for 45 minutes at room temperature. Addition of Amplifier Solution initiates color development.
10. Add 50 µL of Stop Solution to each well. Addition of Stop Solution does not affect 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.).

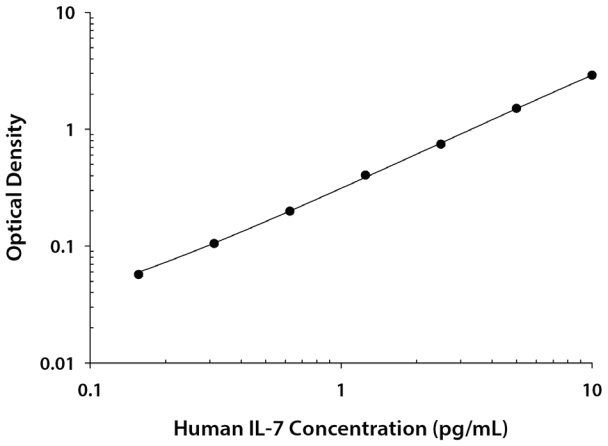
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IL-7 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

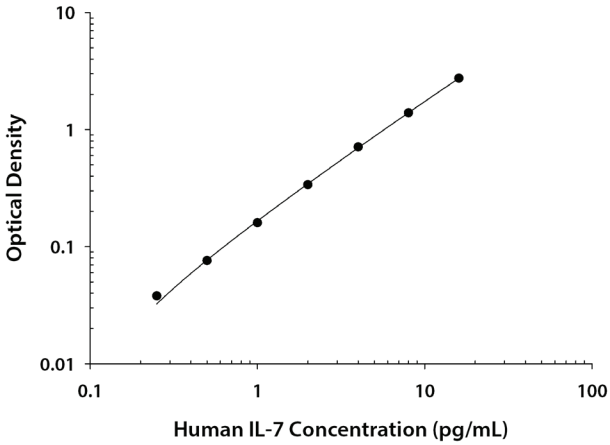
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.091 0.089	0.090	—
0.156	0.150 0.144	0.147	0.057
0.313	0.188 0.202	0.195	0.105
0.625	0.280 0.298	0.289	0.199
1.25	0.485 0.504	0.494	0.404
2.5	0.814 0.854	0.834	0.744
5	1.584 1.603	1.594	1.504
10	2.827 3.153	2.990	2.900

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.063 0.068	0.066	—
0.25	0.103 0.105	0.104	0.038
0.5	0.142 0.143	0.143	0.077
1	0.224 0.227	0.226	0.160
2	0.403 0.404	0.404	0.338
4	0.775 0.777	0.776	0.710
8	1.459 1.453	1.456	1.390
16	2.701 2.913	2.807	2.741

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	0.50	1.49	6.86	0.78	2.13	8.81
Standard deviation	0.04	0.14	0.61	0.08	0.16	0.64
CV (%)	8.0	9.4	8.9	10.3	7.5	7.3

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	0.46	5.79	11.18	0.51	6.38	12.95
Standard deviation	0.03	0.19	0.44	0.06	0.50	1.30
CV (%)	6.5	3.3	3.9	11.8	7.8	10.0

RECOVERY

The recovery of human IL-7 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	103	89-113%
Serum (n=5)	100	91-113%
EDTA plasma (n=5)	112	83-132%
Citrate plasma (n=5)	100	92-110%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-7 were serially diluted with 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)	Citrate plasma (n=5)
1:2	Average % of Expected	96	102	97	99
	Range (%)	88-101	98-107	92-102	98-105
1:4	Average % of Expected	99	89	103	95
	Range (%)	85-111	82-105	92-114	85-103
1:8	Average % of Expected	104	102	103	101
	Range (%)	92-114	94-109	98-109	89-112

SENSITIVITY

The minimum detectable dose (MDD) of human IL-7 is typically less than 0.1 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 highly purified *E. coli*-expressed recombinant human IL-7 produced at R&D Systems®.

The NIBSC/WHO IL-7 Reference Reagent 90/530, which is intended as a potency standard, was evaluated in this kit. The dose response curve of this Reference Reagent parallels the Quantikine® HS standard curve. To convert sample values obtained with the Quantikine® HS Human IL-7 kit to approximate NIBSC units, use the equation below.

NIBSC/WHO (90/530) approximate value (U/mL) = 0.19 x Quantikine® HS Human IL-7 value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-7 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=32)	2.51	0.267-9.80	2.64
EDTA plasma (n=37)	2.99	0.921-7.82	1.37
Citrate plasma (n=38)	1.90	0.664-4.03	0.888

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured for 1, 3, and 5 days. All measured below the lowest human IL-7 standard, 0.156 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-7.

The factors listed below were prepared at 10 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range recombinant human IL-7 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-6 R
IL-7 Rα
IL-8
IL-9
IL-10
IL-11

Recombinant mouse:

IL-7

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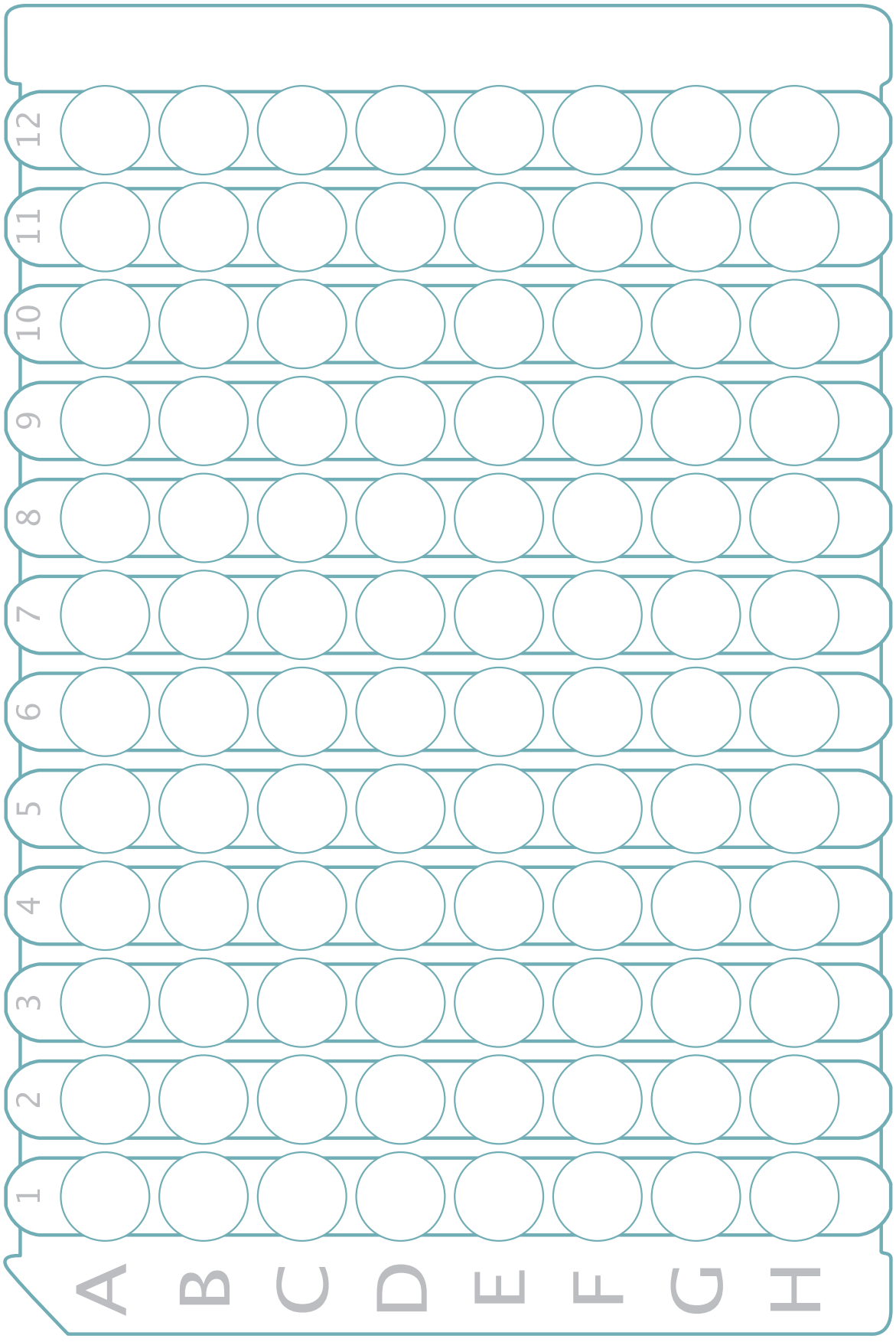
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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