Quantikine® HS ELISA

Human IL-4 Immunoassay

Catalog Number HS400
SS400
PHS400

For the quantitative determination of human Interleukin 4 (IL-4) concentrations in cell culture supernates and serum.

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 4 (IL-4) is a pleiotropic cytokine produced primarily by activated T lymphocytes, mast cells and basophils (1-3). IL-4 has multiple immune response-modulating functions on a variety of cell types. It is an important regulator of isotype switching, inducing IgE production in B lymphocytes. It is an important modulator of the differentiation of precursor T helper cells to the Th2 subset that mediates humoral immunity and modulates antibody production. In addition, IL-4 has also been shown to have anti-tumor activity both *in vivo* and *in vitro* (1-3).

The sequence of human IL-4 cDNA predicts a 153 amino acid (aa) residue precursor protein containing a 24 aa residue signal peptide that is cleaved to form the mature protein (4). At the amino acid sequence level, mature human IL-4 is approximately 50% identical to mouse IL-4, and there is no species cross-reactivity between the two proteins (1, 2). Human IL-4 also shares approximately 30% amino acid sequence identity to human IL-13, and the two cytokines exhibit overlapping biological activities (5, 6). The gene for IL-4 has been mapped to human chromosome 5q, in close proximity to the genes for IL-3, IL-5, IL-13, and GM-CSF (1, 2).

The biological effects of IL-4 are mediated by specific cell surface receptor complexes. One type of functional IL-4 receptor complex consists of the IL-4-binding subunit (IL-4 R) and a second chain, designated the common γ_c chain because it has also been identified as a component of the receptor complexes for IL-2, IL-7, IL-9, and IL-15 (7-9). A second type of functional IL-4 receptor complex, consisting of the IL-4 R and IL-13 Rα, has also been proposed (10, 11). Although IL-4 R does not bind IL-13 directly, it has been shown to complex with the low-affinity IL-13 Rα to form the functional high-affinity receptor complex for IL-13 (11, 12). In addition to the membrane-bound form of IL-4 R, a naturally occurring soluble form of IL-4 R has been identified in human and mouse biological fluids and in mouse cell culture supernates (13-15). Soluble IL-4 R has been to shown to bind IL-4 with high affinity in solution.

The Quantikine® HS Human IL-4 Immunoassay is a 6.5 hour solid phase ELISA designed to measure human IL-4 levels in cell culture supernates and serum. It contains *E. coli*-expressed recombinant human IL-4 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant human IL-4 accurately. Results obtained using natural human IL-4 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-4.

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-4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-4 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-4 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-4 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- Although this kit has been designed to eliminate matrix problems, some samples may exist that give falsely elevated values when assayed neat. If either serum or cell culture supernate 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.
- Samples containing high levels of triglycerides may interfere with the performance of this 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.
- 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.
- 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 #	CATALOG # HS400	CATALOG # SS400	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-4 HS Microplate	890399	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-4.	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-4 HS Standard	890258	1 vial	6 vials	Recombinant human IL-4 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Store for up to 1 month at 2-8 °C.*
Human IL-4 HS Conjugate	890260	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-4, conjugated to alkaline phosphatase with preservatives.	
Assay Diluent RD1-6	895158	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent HD5N	895147	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6U	895148	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum samples. May contain a precipitate.*	
Wash Buffer Concentrate	895188	1 vial	6 vials	100 mL/vial of a 10-fold concentrated solution of buffered surfactant with preservatives. *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.	
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.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

HS400 contains sufficient materials to run an ELISA on one 96 well plate.
SS400 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PHS400). 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 autowasher.
- 500 mL graduated cylinder for preparation of Wash Buffer.
- Test tubes for dilution of standards.
- Human IL-4 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 MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly lipemic samples are not suitable for measurement of human IL-4 with this assay.*

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.

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).*

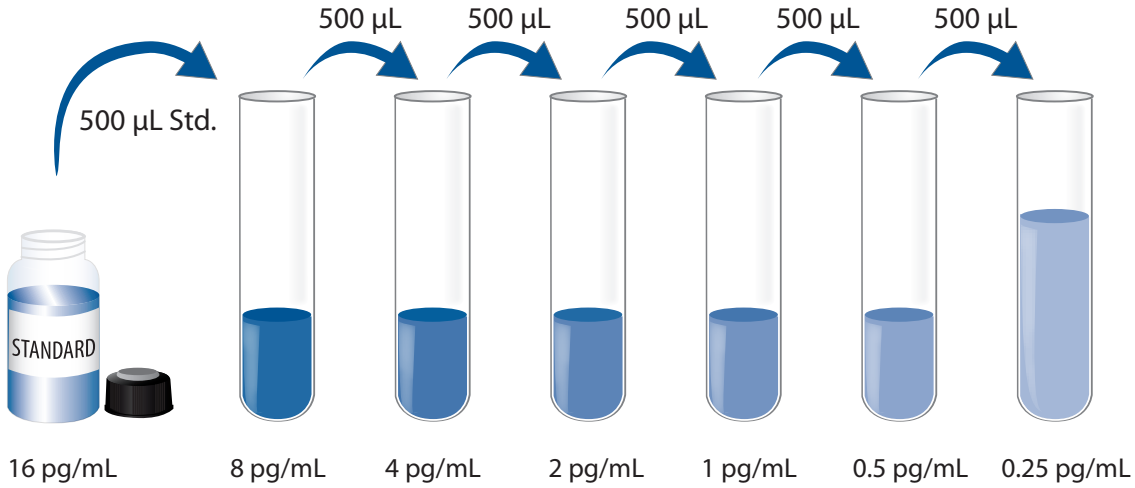
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

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.

Human IL-4 HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-4 HS Standard with Calibrator Diluent HD5N (*for cell culture supernate samples*) or Calibrator Diluent RD6U (*for serum samples*). *Calibrator Diluent RD6U may contain a precipitate. Mix well before and during use.* 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 the appropriate Calibrator Diluent HD5N (*for cell culture supernate samples*) or Calibrator Diluent RD6U (*for serum samples*) 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-4 HS Standard (16 pg/mL) serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

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

1. Prepare all reagents, samples, 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 50 µL of Assay Diluent RD1-6 to each well. *Assay Diluent RD1-6 may have a precipitate present. Mix well before and during its use.*
4. Add 200 µL of standard, sample, or control per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Wash
   a. Remove liquid from the wells by aspirating or inverting the plate and decanting the contents.
   b. Remove excess liquid by grasping the plate firmly and smartly rapping the inverted plate 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 aspirating or inverting the plate and decanting the contents.
   e. Repeat steps b, c, and d 3 times for a total of 4 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-4 HS 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 1 hour 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 30 minutes at room temperature. **Note:** *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.).

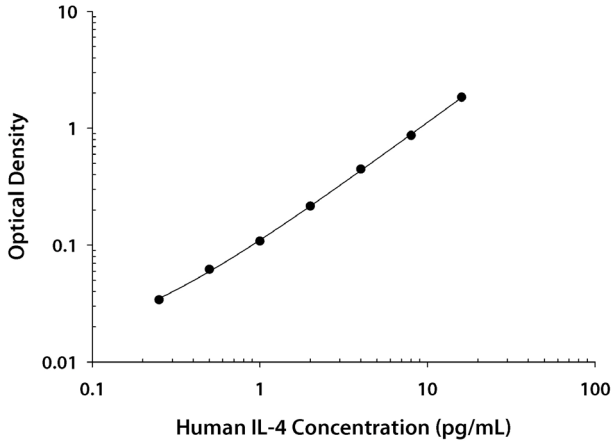
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

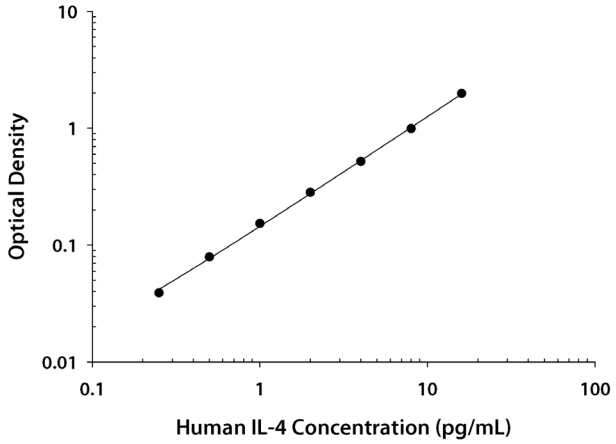
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.078 0.087	0.083	—
0.25	0.114 0.118	0.116	0.033
0.5	0.140 0.148	0.144	0.061
1	0.186 0.195	0.190	0.107
2	0.296 0.300	0.298	0.215
4	0.522 0.536	0.529	0.446
8	0.938 0.961	0.950	0.867
16	1.907 1.939	1.923	1.840

SERUM ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.085 0.086	0.086	—
0.25	0.124 0.126	0.125	0.039
0.5	0.165 0.166	0.166	0.080
1	0.234 0.245	0.240	0.154
2	0.362 0.377	0.370	0.284
4	0.601 0.609	0.605	0.519
8	1.064 1.093	1.078	0.992
16	2.067 2.074	2.070	1.984

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)	0.68	3.79	12.3	0.61	3.56	11.1
Standard deviation	0.05	0.30	0.94	0.06	0.29	0.90
CV (%)	7.4	7.9	7.6	9.8	8.1	8.1

SERUM ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	1.75	5.56	10.7	0.59	3.32	10.7
Standard deviation	0.05	0.28	0.29	0.06	0.19	0.79
CV (%)	2.9	5.0	2.7	10.2	5.7	7.4

RECOVERY

The recovery of human IL-4 was determined by spiking to levels throughout the range of the assay in various matrices.

Sample Type	Average % Recovery	Range
Cell culture media (n=9)	106	87-118%
Serum (n=4)	102	95-108%

SENSITIVITY

Eleven assays were evaluated and the minimum detectable dose (MDD) of human IL-4 ranged from 0.03-0.22 pg/mL. The mean MDD was 0.11 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.

LINEARITY

To assess linearity of the assay, the following samples were spiked with high concentrations of human IL-4, diluted with the appropriate calibrator diluent, and assayed.

		Cell culture media (n=9)	Serum (n=4)
1:2	Average % of Expected	90	100
	Range (%)	85-94	96-104
1:4	Average % of Expected	90	101
	Range (%)	85-98	98-103
1:8	Average % of Expected	92	96
	Range (%)	85-108	90-99
1:16	Average % of Expected	88	97
	Range (%)	83-96	96-98

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-4 produced at R&D Systems®.

The NIBSC/WHO IL-4 1st International Standard 88/656, which was intended as a potency standard, was evaluated in this kit. This standard is an *E. coli*-expressed recombinant human IL-4. The interim reference material parallels the Quantikine® HS standard curve. To convert sample values obtained with the Quantikine® HS Human IL-4 kit to approximate NIBSC 88/656 International Units, use the equation below.

NIBSC/WHO (88/656) approximate value (IU/mL) = 0.0163 x Quantikine® HS IL-4 value (pg/mL)

SAMPLE VALUES

Serum - Sixty serum samples from apparently healthy volunteers were evaluated for the presence of human IL-4 in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human IL-4 standard, 0.25 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells (2 x 10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured stimulated with 10 ng/mL PMA and 0.1 or 1.0 µg/mL calcium ionophore. Aliquots of the cell culture supernates were removed on day 7 and assayed for levels of human IL-4.

Stimulant	Day 7 (pg/mL)
0.1 µg/mL calcium ionophore	12.2
1.0 µg/mL calcium ionophore	17.5

SPECIFICITY

This assay recognizes natural and recombinant human IL-4.

The factors listed below were prepared at 50 ng/mL in the appropriate calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL or 50 ng/mL in a mid-range recombinant human IL-4 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Common γ chain
GM-CSF
gp130
IL-2 Rα
IL-3
IL-3 Rα
IL-3 Rβ
IL-5
IL-5 Rα
IL-6 R
IL-7
IL-13
IL-13 Rα1
IL-13 Rα2

Recombinant mouse:

Common γ chain
IL-3
IL-4
IL-4 R
IL-4 Rα
IL-5
IL-6
IL-7
IL-13
IL-13 Rα1
IL-13 Rα2

Other recombinants:

bovine IL-4
canine IL-4
cotton rat IL-4
equine IL-4
feline IL-4
porcine IL-4
rabbit IL-4
rat IL-4
rhesus macaque IL-4

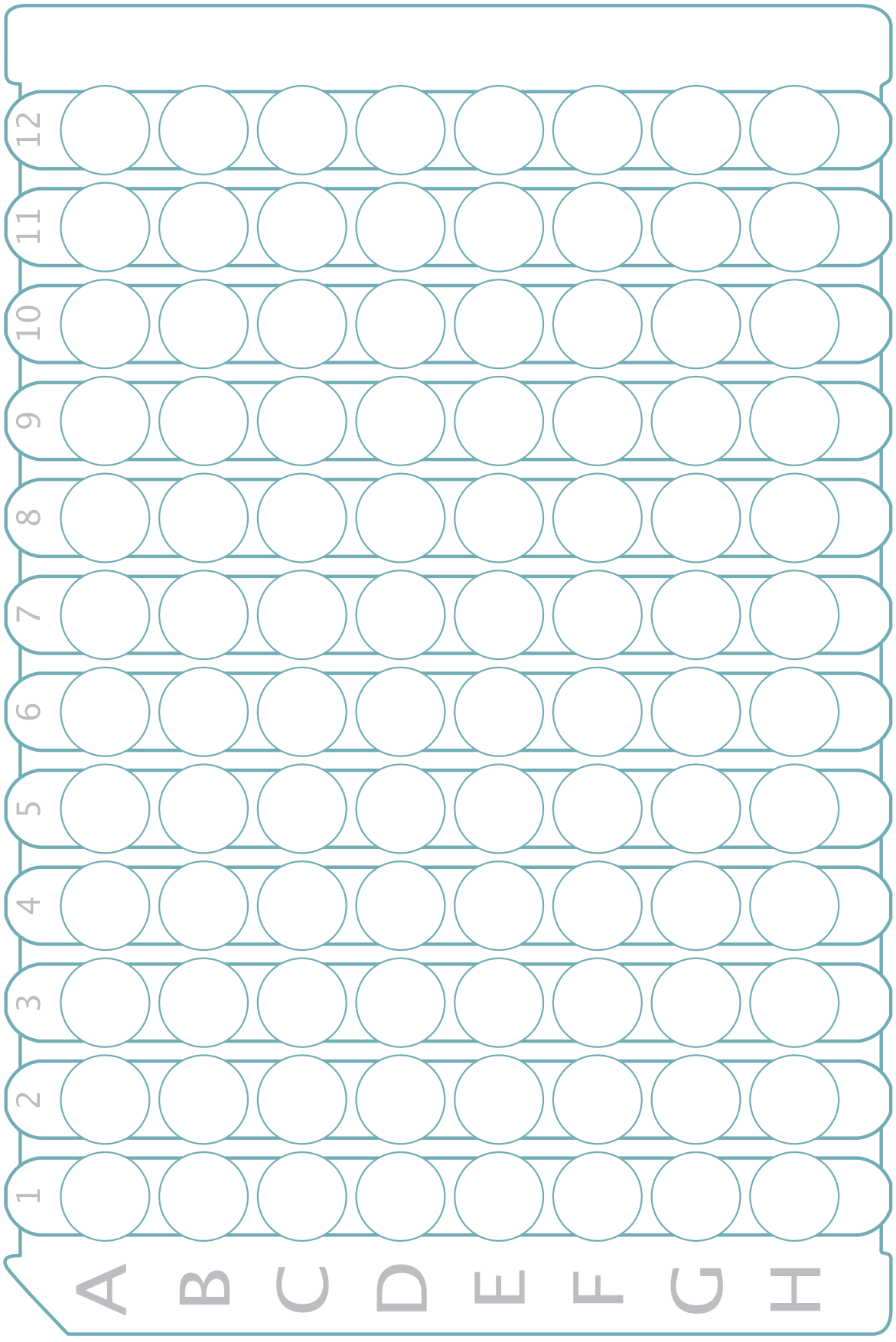
Recombinant human IL-4 Rα interferes at concentrations ≥ 10,000 pg/mL.

REFERENCES

1. Howard, M. and N. Harada (1994) in *Guidebook to Cytokines and Their Receptors*, N.A. Nicola ed., Oxford Univ. Press, New York, p. 44.
2. Banchereau, J. and M.E. Rybak (1994) in *The Cytokine Handbook*, 2nd Ed., A. Thomson ed., Academic Press, New York, p. 99.
3. Yokota, T. *et al.* (1990) in *Peptide Growth Factors and Their Receptors I*, Sporn, M.B. and A.B. Roberts eds., Springer-Verlag, New York, p. 577.
4. Yokata T. *et al.* (1986) Proc. Natl. Acad. Sci. USA **83**:5894.
5. McKenzie, A.N.J. and G. Zurawski (1994) in *Guidebook to Cytokines and Their Receptors*, N.A. Nicola ed., Oxford Univ. Press, New York, p. 92.
6. Zurawski, G. and J.E. de Vries (1994) Immunol. Today **15**:19.
7. Park, L.S. (1994) in *Guidebook to Cytokines and Their Receptors*, N.A. Nicola ed., Oxford Univ. Press, New York, p. 47.
8. Noguchi, M. *et al.* (1993) Science **262**:1877.
9. Kondo, M. *et al.* (1993) Science **262**:1874.
10. Hilton, D.J. *et al.* (1996) Proc. Natl. Acad. Sci. USA **93**:497.
11. Lin, J-X. *et al.* (1995) Immunity **2**:331.
12. Zurawski, S.M. *et al.* (1995) J. Biol. Chem. **270**:13869.
13. Fanslow, W.C. *et al.* (1990) Cytokine **2**:398.
14. Fernandez-Botran, R. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:4202.
15. Ruhl, S. *et al.* (1993) Cytokine **5**:144.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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