Quantikine® ELISA

Human IL-1ra/IL-1F3 Immunoassay

Catalog Number DRA00B
SRA00B
PDRA00B

For the quantitative determination of human Interleukin 1 receptor antagonist (IL-1ra) 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-1 receptor antagonist (IL-1ra; also known as IL-1F3) is a 22-25 kDa member of the IL-1 family of cytokines. Currently, there are 11 family members (IL-1F1-F11), nine of which form an IL-1 gene cluster on human Ch2 (1-3). Each IL-1 family member contains an IL-1 fold. This fold is generated by 12 packed β-sheets that interact to form a β-trefoil structure. Little amino acid (aa) homology is required to achieve this structure, and this explains the low aa identity among IL-1 family members. IL-1ra is a pure cytokine receptor antagonist that has no signal transduction-initiating activity (4). It is an acute phase protein that exists to dampen inflammation. IL-1(β) is initially produced by monocytes in response to a variety of stimuli. Circulating IL-1 then binds to widely expressed IL-1 type I receptors (IL-1 RI) and initiates a number of pro-inflammatory events. On endothelial cells (EC), IL-1 induces PGE_2 and IL-6 release, generating fever, thrombocytosis, and hepatic acute phase protein production. In synovial joints, IL-1 induces chondrocyte NO production, an event that leads to reduced collagen synthesis and chondrocyte apoptosis. Finally, IL-1 increases neutrophil counts, both in blood and tissue, and thus is able to promote a pro-inflammatory environment in multiple locations (5-8). IL-1ra blocks IL-1 action through competitive inhibition. More correctly, although IL-1ra fills the IL-1 binding site in IL-1 RI, it is also unable to orchestrate the creation of a signal-transducing IL-1 RI:IL-1 R Accessory protein (IL-1 R AcP) heterodimer complex. Effective IL-1ra concentrations are generally 100-fold greater than local IL-1 concentrations. This is because the IL-1ra half-life is but 6 minutes, and very few IL-1 type I receptors need to be engaged by IL-1 to elicit a cellular response (5, 7, 9).

Human IL-1ra is synthesized as a 177 aa precursor that contains a 25 aa signal sequence and a 152 aa mature region (10, 11). Although it contains an IL-1 cytokine fold, it apparently lacks two structural motifs that allow for activation of the IL-1 receptor heterodimer. First, and following binding to IL-1 RI, the presence of Ile 51-His 54 and Lys 145 of the mature molecule preclude recruitment of IL-1 R AcP. Second, there is no identifiable C-terminal lectin segment that is hypothesized to help recruit an accessory signaling component (1, 12, 13). Mature human IL-1ra is 77% and 82% aa identical to mouse and canine IL-1ra, respectively, and human IL-1ra inhibits IL-1 activity on mouse cells (10). A number of cell types express IL-1ra, including monocytes (11), Sertoli cells (14), hepatocytes (15), adipocytes (16), synovial fibroblasts (17), mast cells (18), pancreatic β-cells (19), and intestinal epithelial cells (20). There are at least three intracellular IL-1ra isoforms (icIL-1ra1, 2, and 3). All show N-terminal variation, and all contain amino acids 35-177 of the secreted precursor (21-23). Intracellular IL-1ra1 is of particular interest, because it is reported to be "secreted" by endothelial cells and binds to the IL-1 RI in an antagonist fashion (23-25). Intracellular IL-1ra1 is 159 aa in length and shows a 3 aa substitution for the first 21 aa's of the signal sequence of IL-1ra (21).

The Quantikine® Human IL-1ra/IL-1F3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-1ra in cell culture supernates, serum, and plasma. It contains *E. coli*-derived recombinant human IL-1ra as well as antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-1ra. Results obtained using natural human IL-1ra 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 human IL-1ra.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-1ra has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1ra present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-1ra is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-1ra 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.
- 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, however, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DRA00B	CATALOG # SRA00B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-1ra Microplate	893205	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-1ra.	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-1ra Standard	893207	1 vial	6 vials	Recombinant human IL-1ra in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	May be stored for up to 1 month at ≤ -20 °C.* Avoid repeated freeze-thaw cycles.
Human IL-1ra Conjugate	893206	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-1ra conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1S	895137	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-33	895813	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples. Use diluted 1:2 in this assay.*	
Calibrator Diluent RD6-3	895165	1 vial	6 vials	21 mL/vial of buffered animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DRA00B contains sufficient materials to run an ELISA on one 96 well plate.
SRA00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDRA00B). 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 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.
- 50 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards.
- Human IL-1ra Controls (optional; R&D Systems®, Catalog # QC25).

PRECAUTIONS

IL-1ra 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 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of IL-1ra are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

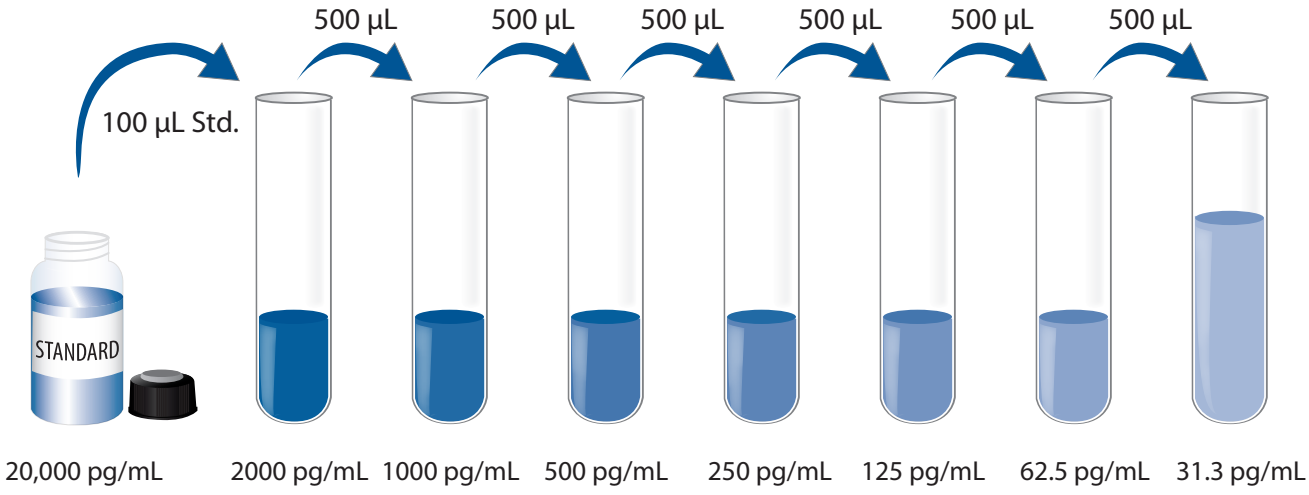
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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

Calibrator Diluent RD5-33 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD5-33 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-33 (diluted 1:2).

Human IL-1ra Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-1ra Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-33 (diluted 1:2) (*for cell culture supernate samples*) or Calibrator Diluent RD6-3 (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard 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: *High concentrations of IL-1ra 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, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1S to all wells.
4. Add 100 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human IL-1ra Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. **Protect from light.**
   For Cell Culture Supernate samples: Incubate for **20 minutes** at room temperature.
   For Serum/Plasma samples: Incubate for **30 minutes** at room temperature.
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

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

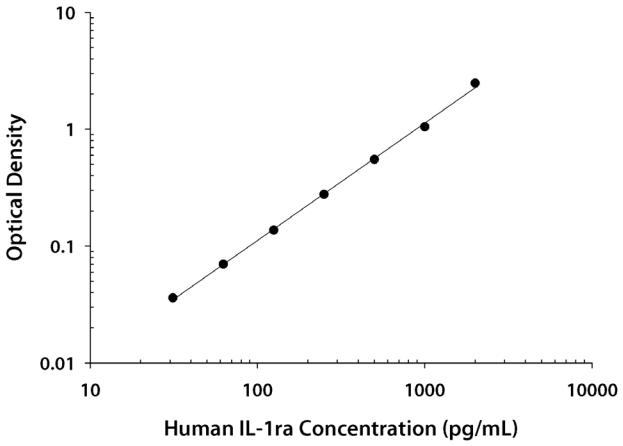
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-1ra concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

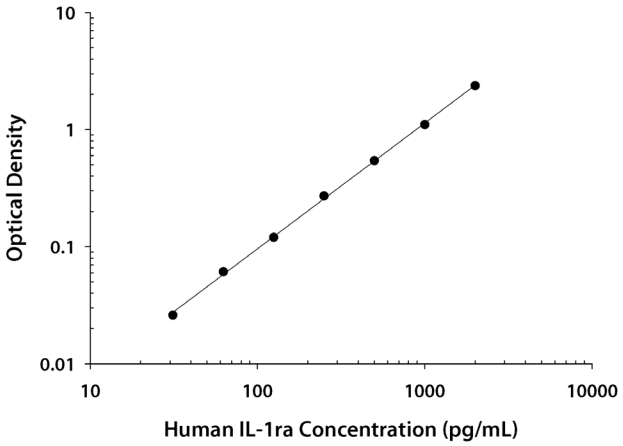
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.025	0.024	—
31.3	0.060 0.060	0.060	0.036
62.5	0.093 0.094	0.094	0.070
125	0.158 0.164	0.161	0.137
250	0.293 0.309	0.301	0.277
500	0.564 0.585	0.575	0.551
1000	1.052 1.088	1.070	1.046
2000	2.459 2.537	2.498	2.474

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.030	0.029	—
31.3	0.054 0.055	0.055	0.026
62.5	0.085 0.095	0.090	0.061
125	0.139 0.159	0.149	0.120
250	0.281 0.319	0.300	0.271
500	0.545 0.598	0.572	0.543
1000	1.075 1.186	1.131	1.102
2000	2.434 2.368	2.401	2.372

PRECISION

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

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

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	64.9	538	1343	56.2	469	1243
Standard deviation	3.67	22.1	55.7	5.79	31.9	70.4
CV (%)	5.7	4.1	4.1	10.3	6.8	5.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	66.9	607	1627	82.5	646	1663
Standard deviation	4.88	30.2	60.0	9.04	51.4	111
CV (%)	7.3	5.0	3.7	11.0	8.0	6.7

RECOVERY

The recovery of human IL-1ra spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernate (n=4)	106	96-115%
Serum (n=4)	90	81-99%
EDTA plasma (n=4)	86	66-102%
Heparin plasma (n=4)	93	82-106%

SENSITIVITY

Fifty-two assays were evaluated and the minimum detectable dose (MDD) of human IL-1ra ranged from 2.2-18.3 pg/mL. The mean MDD was 6.3 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 the linearity of the assay, samples containing high concentrations of human IL-1ra were serially diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	97	107	105	104
	Range (%)	91-101	106-108	95-113	94-113
1:4	Average % of Expected	100	111	103	103
	Range (%)	95-106	102-119	86-116	93-117
1:8	Average % of Expected	105	113	112	108
	Range (%)	100-110	98-127	96-123	102-122
1:16	Average % of Expected	101	123	123	118
	Range (%)	99-103	110-135	103-143	104-132

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-1ra produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-1ra 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=35)	360	181-1327	214
EDTA plasma (n=35)	230	105-1062	177
Heparin plasma (n=35)	309	160-1193	194

Cell Culture Supernates:
Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-1ra.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	1286	49,621
Stimulated	9998	18,803

Jurkat human acute T cell leukemia cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed and assayed for human IL-1ra. No detectable levels were observed.

IMR-90 human lung fibroblast cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed and assayed for human IL-1ra. No detectable levels were observed.

THP-1 human acute monocytic leukemia cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum and 50 µM β-mercaptoethanol. An aliquot of the cell culture supernate was removed, assayed for human IL-1ra, and measured 1464 pg/mL.

U937 human histiocytic lymphoma cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human IL-1ra, and measured 134 pg/mL.

HEK293 human embryonic kidney cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed after 5 days and assayed for human IL-1ra. No detectable levels were observed.

HepG2 human hepatocellular carcinoma cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human IL-1ra, and measured 597 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-1ra.

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 human IL-1ra control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-1α
IL-1β
IL-1F7
IL-1 R2
IL-1 R3
IL-1 R4
IL-1 R6
IL-1 R9
IL-18 Rα
IL-18 Rβ
IL-18 BPα
SIGIRR

Recombinant mouse:

IL-1α
IL-1β
IL-1 R2
IL-1 R4
IL-18
IL-18 BPδ
RP105
SIGRR

Recombinant rat:

IL-1α
IL-1β
IL-1ra
IL-1 R6
IL-18

Recombinant porcine:

IL-1α
IL-1β
IL-18

Other recombinants:

cotton rat IL-1α
cotton rat IL-1β
feline IL-1β

Recombinant mouse and recombinant porcine IL-1ra show 0.4% cross-reactivity at concentrations > 10 ng/mL.

Recombinant human IL-1 RI interferes at concentrations > 2.0 ng/mL.

Recombinant mouse IL-1 RI interferes at concentrations > 10 ng/mL.

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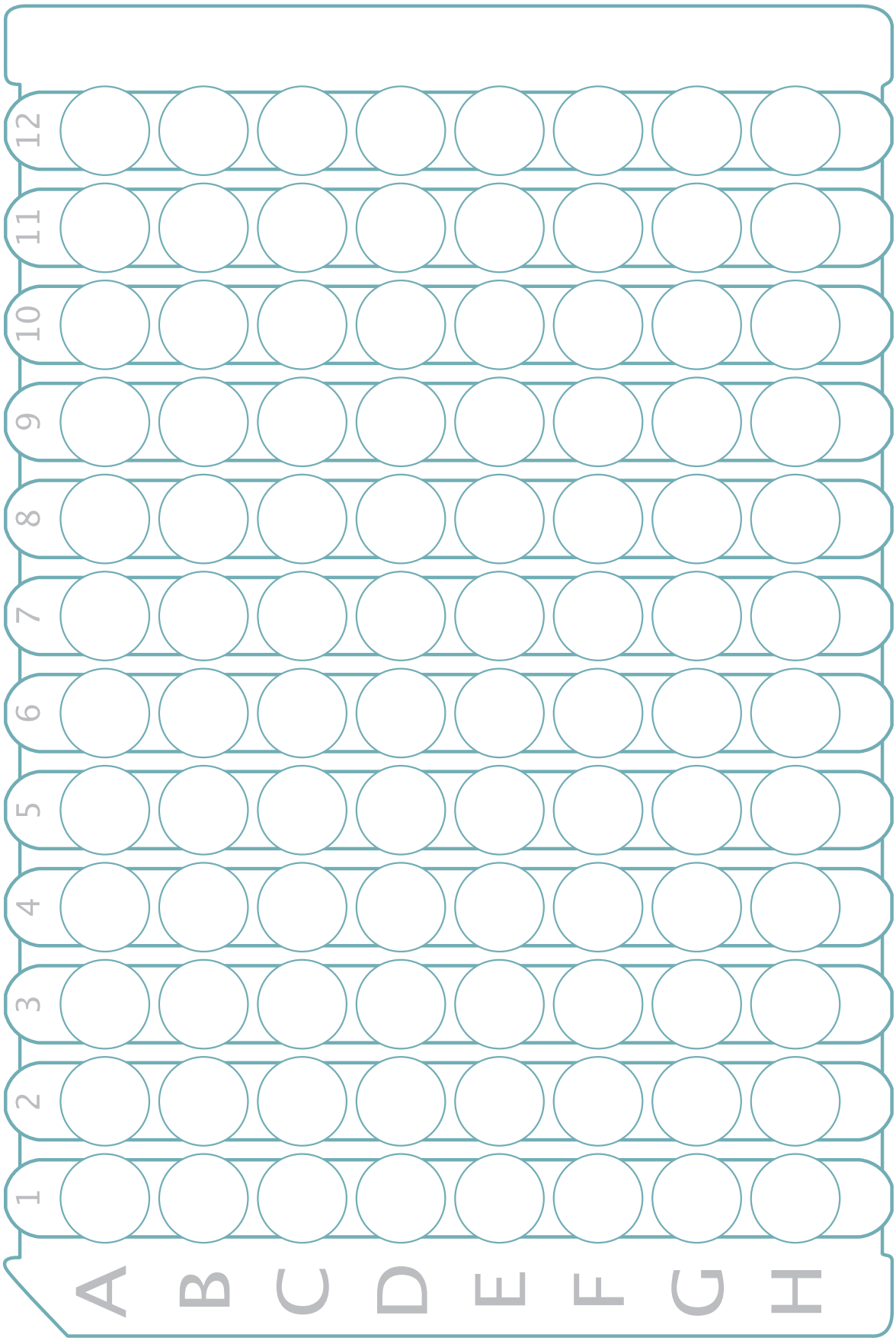
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES