Quantikine® HS ELISA

Human IL-1β/IL-1F2 Immunoassay

Catalog Number HSLB00D
SSLB00D
PHSLB00D

For the quantitative determination of human Interleukin 1 beta (IL-1β) concentrations in serum, plasma, and saliva.

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

The Interleukin 1 (IL-1) family of proteins consists of the classic members IL-1α, IL-1β, and IL-1ra, plus IL-18, IL-33 and IL-1F5-F10. IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (1, 2). IL-1 is typically not produced by unstimulated cells of healthy individuals with the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system. However, in response to inflammatory agents, infections, or microbial endotoxins, a dramatic increase in the production of IL-1 by macrophages and various other cell types is seen. IL-1β plays a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. Inappropriate or prolonged production of IL-1 has been implicated in a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases (3-6).

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid level. Both are synthesized as 31 kDa precursors that are subsequently cleaved into mature proteins of approximately 17.5 kDa (7, 8). Cleavage of the IL-1β precursor by Caspase-1/ICE is a key step in the inflammatory response (3, 9). Neither IL-1α nor IL-1β contains a typical hydrophobic signal peptide (10-12), but evidence suggests that these factors can be secreted by non-classical pathways (13-15). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (16). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (14, 17). Both unprocessed and mature forms of IL-1β are exported from the cell.

IL-1α and IL-1β exert their effects through immunoglobulin superfamily receptors that additionally bind IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (18, 19). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (20). The two IL-1 receptor types show approximately 28% homology in their extracellular domains but differ significantly in that the type II receptor has a cytoplasmic domain of only 29 amino acids (aa), whereas the type I receptor has a 213 aa cytoplasmic domain. IL-1 RII does not appear to signal in response to IL-1 and may function as a decoy receptor that attenuates IL-1 function (21). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (22). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (23, 24). Soluble forms of both IL-1 RI and IL-1 RII have been detected in human plasma, synovial fluids, and the conditioned media of several human cell lines (25, 26). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (27).

The Quantikine HS Human IL-1β Immunoassay is a 4 hour solid phase ELISA designed to measure human IL-1β levels in serum, plasma, and saliva. It contains *E. coli*-expressed recombinant human IL-1β and antibodies raised against the recombinant factor and has been shown to accurately quantitate recombinant human IL-1β. Results obtained using natural IL-1β showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit standards. These results indicate that the Quantikine HS Immunoassay kit can be used to determine relative mass values for natural human IL-1β. Reports indicate that ELISA kits calibrated using mature IL-1β as a standard will detect, but considerably underestimate, the unprocessed IL-1β precursor present in samples (28, 29). In biological samples other than cell lysates, the precursor form of IL-1β is usually not the predominant form of IL-1β present and, additionally, is not biologically active. Therefore, results obtained using this kit should provide a useful measure of the levels of active IL-1β present in serum, plasma, and saliva.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for human IL-1β is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin is added to the wells. After washing away any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-1β bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in 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 ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Assay precision for plasma samples can be further improved with additional centrifugation of the samples. A five minute centrifugation at 13,000 RPM is recommended for plasma samples.

PRECAUTIONS

IL-1β 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.

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

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # HSLB00D	CATALOG # SSLB00D	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-1β HS Microplate	898340	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-1β.	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-1β HS Standard	898437	2 vials	12 vials	Recombinant human IL-1β in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human IL-1β HS Conjugate	898341	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-1β conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	1 vial	6 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	12 vials	21 mL/vial of a 25-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.	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Streptavidin Polymer-HRP Diluent	898387	1 vial	6 vials	21 mL/vial of a solution with preservatives.	
Streptavidin Polymer-HRP (100X)	898350	1 vial	6 vials	0.3 mL/vial of Streptavidin Polymer-HRP in a buffer with preservative.	
Plate Sealers	N/A	8 strips	48 strips	Adhesive strips.	

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

HSLB00D contains sufficient materials to run an ELISA on one 96 well plate.
SSLB00D (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PHSLB00D). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please 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.
- 1000 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-1β Controls (optional; R&D Systems, Catalog # QC41).

SAMPLE COLLECTION & STORAGE

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

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

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

Note: *Citrate plasma is not validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.
High albumin samples are not suitable for use in this assay.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Saliva samples require a 40-fold dilution. A suggested 40-fold dilution is 10 µL of sample + 390 µL of Calibrator Diluent RD5T.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

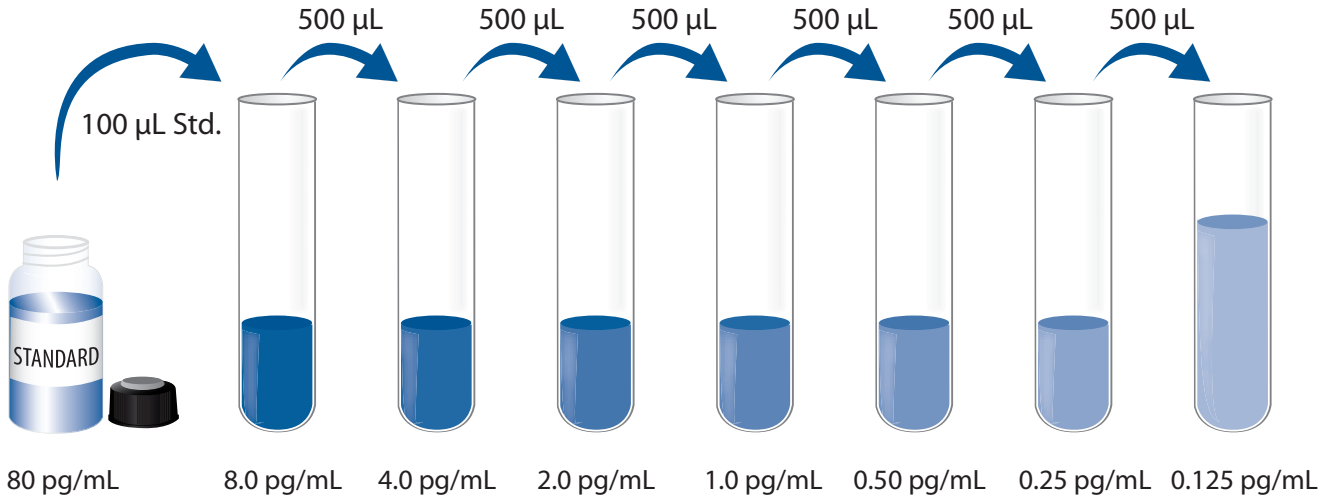
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

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

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

Human IL-1β HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-1β HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 80 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5T into the 8.0 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8.0 pg/mL standard serves as the high standard. Calibrator Diluent RD5T 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: *IL-1β is detectable in saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.*

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 50 µL of Assay Diluent RD1-63 to each well.
4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.

   Note: *If using R&D Systems, Catalog # QC41, pipette within 10 minutes.*
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-1β HS Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 200 µL of Streptavidin Polymer-HRP (1X) to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature on the shaker.
9. Repeat the wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
11. 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.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

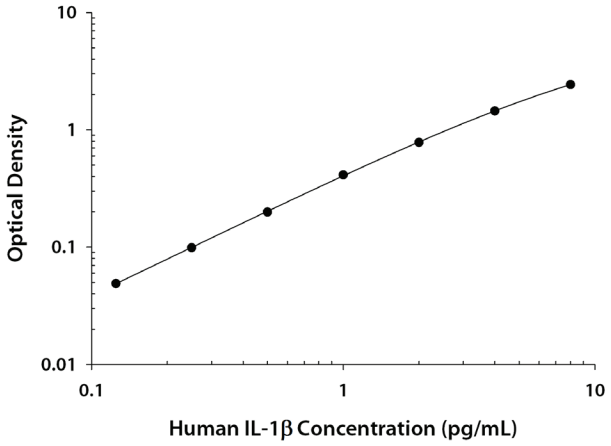
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-1β 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 measured concentrations must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.053 0.060	0.057	—
0.125	0.104 0.108	0.106	0.049
0.25	0.155 0.156	0.156	0.099
0.50	0.255 0.257	0.256	0.199
1.0	0.466 0.473	0.470	0.413
2.0	0.838 0.838	0.838	0.781
4.0	1.490 1.525	1.508	1.451
8.0	2.463 2.511	2.487	2.430

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	0.315	1.30	2.52	0.270	1.36	2.60
Standard deviation	0.014	0.050	0.060	0.029	0.101	0.163
CV (%)	4.4	3.9	2.4	10.7	7.4	6.3

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=4)	91	86-101%
EDTA plasma (n=4)	94	87-106%
Heparin plasma (n=4)	92	89-99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-1β were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)
1:2	Average % of Expected	100	99	98	99
	Range (%)	97-103	95-104	95-102	98-101
1:4	Average % of Expected	106	100	103	103
	Range (%)	104-108	98-102	99-105	100-107
1:8	Average % of Expected	107	103	105	104
	Range (%)	104-113	100-105	103-107	99-109
1:16	Average % of Expected	113	108	108	105
	Range (%)	109-120	103-113	101-115	102-106

SENSITIVITY

Tweny-five assays were evaluated and the minimum detectable dose (MDD) of human IL-1β ranged from 0.014-0.063 pg/mL. The mean MDD was 0.033 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density 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-1β produced at R&D Systems.

The NIBSC/WHO IL-1β 1st International Standard 86/680, which was intended as a potency standard, was evaluated in this kit.

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-1β kit to approximate NIBSC 86/680 values, use the equation below.

NIBSC (86/680) approximate value (IU/mL) = 0.096 x Quantikine Human HS IL-1β value (pg/mL)

Note: *Based on data generated in March 2016.*

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-1β 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=50)	0.357	10	ND-0.606
EDTA plasma (n=50)	0.292	12	ND-0.580
Heparin plasma (n=50)	0.448	14	ND-1.08

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Saliva (n=10)	322	21.9-909	313

SPECIFICITY

This assay recognizes natural and recombinant human IL-1β. This assay also recognizes recombinant rhesus macaque IL-1β (aa 117-269).

The factors listed below were prepared at 80 pg/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 80 pg/mL in a mid-range recombinant human IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-1α
Pro-IL-1β (aa 1-116)
IL-1 ra
IL-1 RAcP/IL-1 R3
IL-1 RI
IL-1 RII
IL-1 RIII
IL-1 Rrp2/IL-1 R6

Other recombinants:

canine IL-1β
cotton rat IL-1β
equine IL-1β
feline IL-1β
guinea pig IL-1β
mouse IL-1β
porcine IL-1β
rabbit IL-1β
rat IL-1β

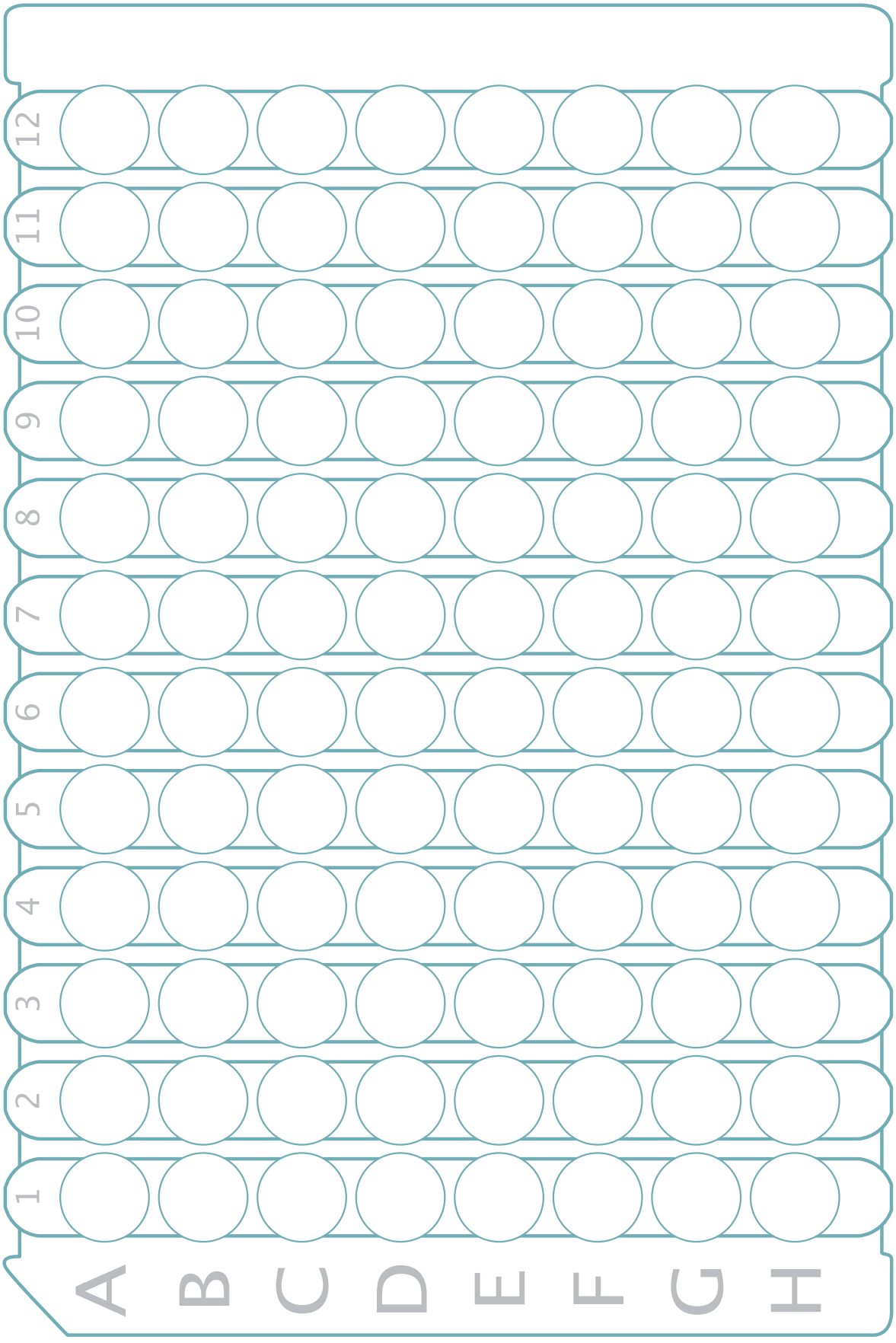
Recombinant human Pro-IL-1β (aa 1-269) cross-reacts approximately 3.9% in this assay.

REFERENCES

1. Sims, J.E and D.E. Smith (2010) Nat. Rev. Immunol. **10**:89.
2. Palomo, J. *et al.* (2015) Cytokine **76**:25.
3. Netea, M.G. *et al.* (2015) Annu. Rev. Immunol. **33**:49.
4. Isoda, K. and F. Ohsuzu (2006) J. Atheroscler. Thromb. **13**:21.
5. Allan, S.M. *et al.* (2005) Nat. Rev. Immunol. **5**:629.
6. Kornman, K.S. (2006) Am. J. Clin. Nutr. **83**:475S.
7. Giri, J.G. *et al.* (1985) J. Immunol. **134**:343.
8. Hazuda, D.J. *et al.* (1990) J. Biol. Chem. **265**:6318.
9. Cerretti, D.P. *et al.* (1992) Science **256**:97.
10. Lomedico, P.T. *et al.* (1984) Nature **312**:458.
11. Auron, P.E. *et al.* (1987) J. Immunol. **138**:1447.
12. March, C.J. *et al.* (1985) Nature **315**:641.
13. Rubartelli, A. *et al.* (1990) EMBO J. **9**:1503.
14. Rubartelli, A. *et al.* (1993) Cytokine **5**:117.
15. Monteleone, M. *et al.* (2015) Cytokine **74**:213.
16. Kurt-Jones, E.A. *et al.* (1985) Proc. Natl. Acad. Sci. **82**:1204.
17. Hazuda, D. *et al.* (1989) J. Biol. Chem. **264**:1689.
18. Urdal, D.L. *et al.* (1988) J. Biol. Chem. **263**:2870.
19. Sims, J.E. *et al.* (1988) Science **241**:585.
20. McMahan, C.J. *et al.* (1991) EMBO J. **10**:2821.
21. Slack, J. *et al.* (1993) J. Biol. Chem. **268**:2513.
22. Greenfeder, S.J. *et al.* (1995) J. Biol. Chem. **270**:13757.
23. Eisenberg, S.P. *et al.* (1990) Nature **343**:341.
24. Carter, D.B. *et al.* (1990) Nature **344**:633.
25. Dayer, J-M. and D. Burger (1994) Eur. Cytokine Netw. **5**:563.
26. Svenson, M. *et al.* (1993) Cytokine **5**:427.
27. Sims, J.E. and S.K. Dower (1994) Eur. Cytokine Netw. **5**:539.
28. Herzyk, D.J. *et al.* (1992) J. Immunol. Methods **148**:243.
29. Dinarello, C.A. (1992) J. Immunol. Methods **148**:255.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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