Quantikine® ELISA

Porcine IL-1β/IL-1F2 Immunoassay

Catalog Number PLB00B

For the quantitative determination of porcine Interleukin 1 beta (IL-1β) 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.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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, IL-36Ra/IL-1F5, IL-36α/IL-1F6, IL-37/IL-1F7, IL-36β/IL-1F8, IL-36γ/IL-1F9, and IL-1F10 (1). IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (2). IL-1 is 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. In response to inflammatory agents, infections, or microbial endotoxins, however, 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 (aa) 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, 14). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (15). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (14, 16). 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 (17, 18). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (19). 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 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 (20). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (21). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (22, 23). 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 (24, 25). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (26).

The Quantikine Porcine IL-1β/IL-1F2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure porcine IL-1β in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mature porcine IL-1β and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant porcine IL-1β. Results obtained using natural porcine IL-1β show dose response curves that are 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 porcine IL-1β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for porcine IL-1β has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any porcine IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for porcine IL-1β is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of porcine IL-1β bound in the initial step. The sample values are then read off the standard curve.

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.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- 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.
- 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.

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
Porcine IL-1β Microplate	890862	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for porcine IL-1β.	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.*
Porcine IL-1β Standard	890863	2 vials of recombinant porcine IL-1β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new Standard and Control for each assay.
Porcine IL-1β Control	890145	2 vials of recombinant porcine IL-1β in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Porcine IL-1β Conjugate	893999	12 mL of a polyclonal antibody against porcine IL-1β conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-14	895180	12 mL of a buffered protein solution with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD6-31	895323	21 mL of diluted animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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.
- 500 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 and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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. Please 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 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 heparin as an anticoagulant. Centrifuge for 20 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: *EDTA plasma is not suitable for use in this assay.*
Citrate plasma has not been validated for use in this assay.
Grossly hemolyzed or icteric samples are not suitable for use in this assay.

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD6-31.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

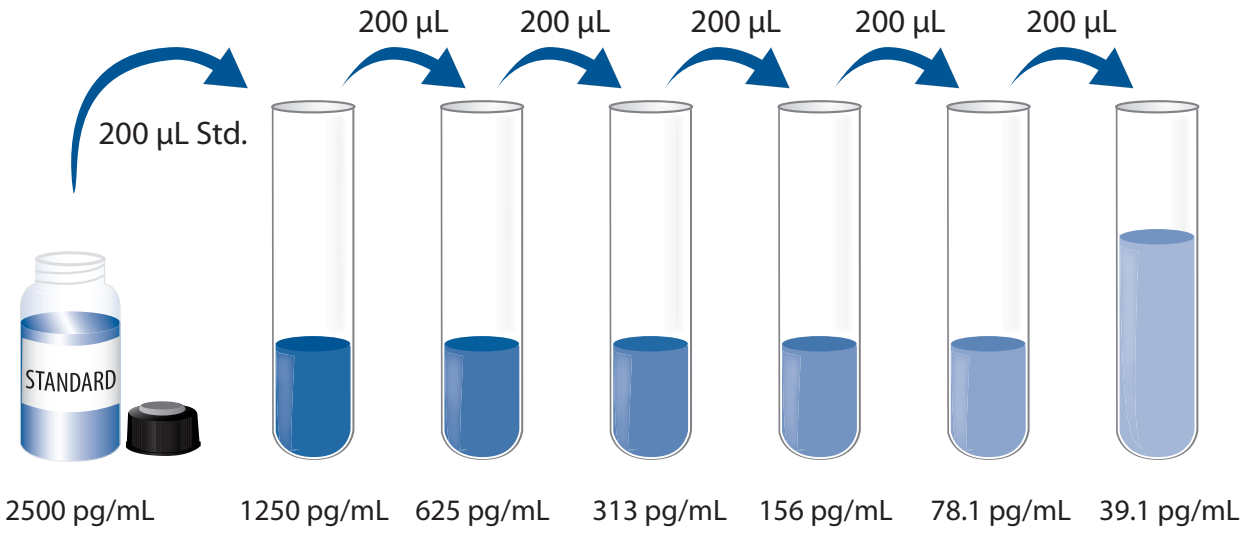
Porcine IL-1β Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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. 100 µL of the resultant mixture is required per well.

Porcine IL-1β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Porcine IL-1β Standard with Calibrator Diluent RD6-31. This reconstitution produces a stock solution of 2500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-31 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Porcine IL-1β Standard (2500 pg/mL) serves as the high standard. Calibrator Diluent RD6-31 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, Control, and samples be assayed in duplicate.

1. Prepare reagents, samples, Control, and standard dilutions as directed by 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 RD1-14 to each well. *Assay Diluent RD1-14 may contain a precipitate. Mix well before and during use.*
4. Add 50 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.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of Porcine IL-1β Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

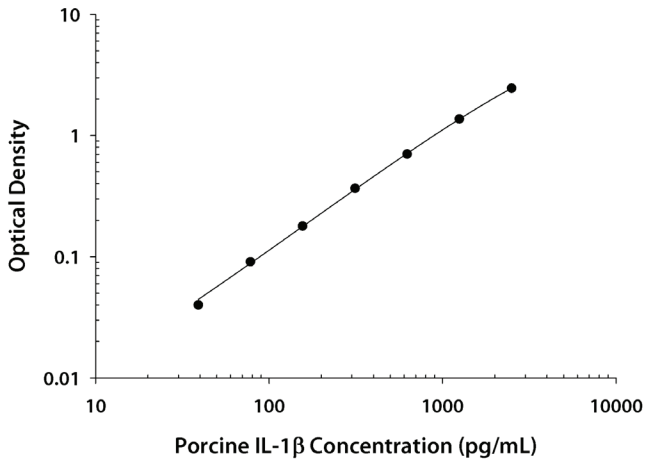
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 porcine 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 concentration read from the standard curve 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	0.083 0.083	0.083	—
39	0.121 0.125	0.123	0.040
78	0.172 0.175	0.174	0.091
156	0.260 0.264	0.262	0.179
313	0.443 0.455	0.449	0.366
625	0.777 0.792	0.785	0.702
1250	1.441 1.464	1.453	1.370
2500	2.519 2.558	2.539	2.456

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)	136	348	780	141	338	783
Standard deviation	9.8	17.0	40.9	12.2	20.5	31.7
CV (%)	7.2	4.9	5.2	8.7	6.1	4.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	102-109%
Serum (n=4)	111	102-117%
Heparin plasma (n=4)	111	98-117%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with porcine IL-1β were serially diluted with Calibrator Diluent and then assayed.

		Cell culture supernates* (n=4)	Serum (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	99	93
	Range (%)	94-102	98-101	92-96
1:4	Average % of Expected	97	101	95
	Range (%)	92-102	98-103	92-98
1:8	Average % of Expected	95	106	93
	Range (%)	79-105	104-110	91-97
1:16	Average % of Expected	102	109	97
	Range (%)	93-109	107-113	89-103

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Thirty assays were evaluated and the minimum detectable dose (MDD) of porcine IL-1β ranged from 2.7-13.6 pg/mL. The mean MDD was 6.7 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 porcine IL-1β produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of porcine IL-1β in the assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=14)	63	21	ND-68
Heparin plasma (n=5)	206	80	ND-366

ND=Non-detectable

Cell Culture Supernates - Porcine peripheral blood lymphocytes (2 x 10^7 cells/mL) were cultured for 1 day in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate and stimulated with 100 ng/mL lipopolysaccharide or 50 ng/mL PMA and 500 ng/mL calcium ionomycin. Aliquots of the cell culture supernates were removed and assayed for levels of porcine IL-1β.

Sample Type	(pg/mL)
PBL Stimulated with LPS	900
PBL Stimulated with PMA + Ca^{2+}	13,700

SPECIFICITY

This assay recognizes natural and recombinant porcine IL-1β.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range porcine IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant porcine:
IL-1α
IL-1 ra
IL-2
IL-4
IL-5
IL-6
IL-8
IL-10
IL-12
IL-18
TNF-α

Recombinant human:
IL-1 RI
IL-1 RII

Natural proteins:
porcine PDGF
porcine TGF-β1

Some cross-reactivity was observed with the following:

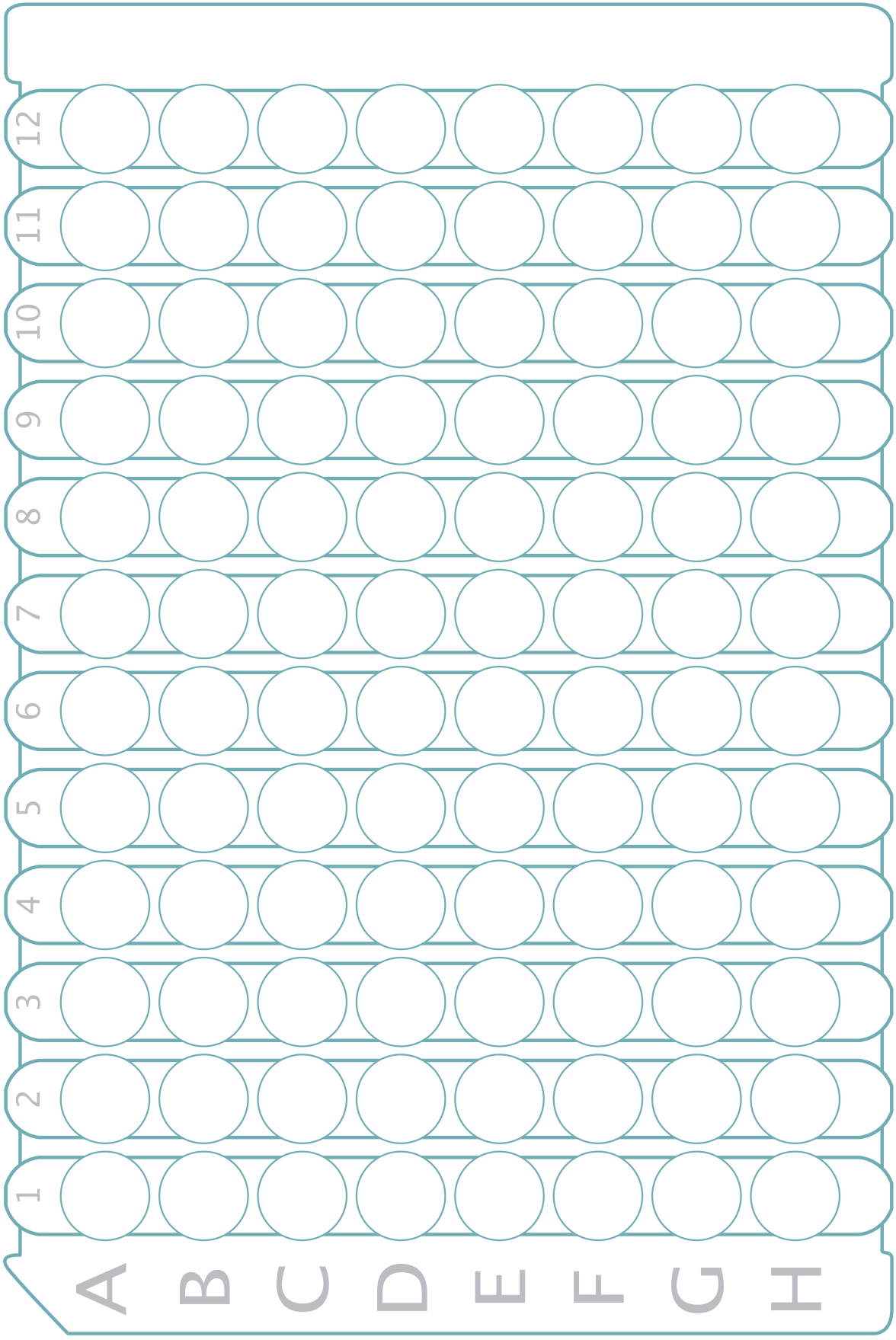
Recombinant Factor	% Cross-reactivity
canine IL-1β	0.4%
equine IL-1β	3.2%
feline IL-1β	0.2%
guinea pig IL-1β	0.4%
human IL-1β	0.05%
mouse IL-1β	0.2%
rat IL-1β	5.8%
rhesus macaque IL-1β	0.1%

REFERENCES

1. Dinarello, C. *et al.* (2010) Nat. Immunol. **11**:973.
2. Sims, J.E and D.E. Smith (2010) Nat. Rev. Immunol. **10**:89.
3. Martinon, F. and J. Tschopp (2007) Cell Death Differ. **14**:10.
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.* (1988) 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. Huether, M.J. *et al.* (1993) Gene **129**:285.
13. Rubartelli, A. *et al.* (1990) EMBO J. **9**:1503.
14. Rubartelli, A. *et al.* (1993) Cytokine **5**:117.
15. Kurt-Jones, E.A. *et al.* (1985) Proc. Natl. Acad. Sci. USA **82**:1204.
16. Hazuda, D. *et al.* (1989) J. Biol. Chem. **264**:1689.
17. Urdal, D.L. *et al.* (1988) J. Biol. Chem. **263**:2870.
18. Sims, J.E. *et al.* (1988) Science **241**:585.
19. McMahan, C.J. *et al.* (1991) EMBO J. **10**:2821.
20. Slack, J. *et al.* (1993) J. Biol. Chem. **268**:2513.
21. Greenfeder, S.J. *et al.* (1995) J. Biol. Chem. **270**:13757.
22. Eisenberg, S.P. *et al.* (1990) Nature **343**:341.
23. Carter, D.B. *et al.* (1990) Nature **344**:633.
24. Dayer, J-M. and D. Burger (1994) Eur. Cytokine Netw. **5**:563.
25. Svenson, M. *et al.* (1993) Cytokine **5**:427.
26. Sims, J.E. and S.K. Dower (1994) Eur. Cytokine Netw. **5**:539.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES