Quantikine™ ELISA

Human IL-18 BPa Immunoassay

Catalog Number DBP180

For the quantitative determination of human Interleukin 18 Binding Protein a (IL-18 BPa) concentrations in cell culture supernates, serum, plasma, and urine.

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-18 binding protein (IL-18 BP) is a secreted glycoprotein that functions as an IL-18 antagonist by preventing the interaction of IL-18 with IL-18 Receptor alpha (1, 2). Full length human IL-18 BP (IL-18 BPa) is a 40 kDa protein that contains one immunoglobulin-like domain (3). Alternative splicing generates isoforms that carry substitutions and truncations at the beginning (IL-18 BPb), in the middle (IL-18 BPd), or following (IL-18 BPc) the Ig-like domain (3, 4). Human IL-18 BPa shares approximately 63% amino acid sequence identity with full-length mouse and rat IL-18 BPa. IL-18 interacts with the widely expressed IL-18 Rα which then recruits the signaling subunit IL-18 Rβ (5, 6). Human IL-18 BPa binds to IL-18 with high affinity and blocks the IL-18 induced inflammatory response in both human and mouse (3, 7). IL-18 BPc also neutralizes IL-18 bioactivity, while IL-18 BPb and IL-18 BPd are much less effective (4). IL-18 BP additionally binds to IL-37b/IL-1F7b, forming a complex which shows enhanced neutralization of IL-18 (8).

IL-18 is secreted by a variety of cell types including macrophages, dendritic cells, and epithelial cells (1, 2). It promotes both Th1 and Th2 inflammation, memory $CD8^+$ T cell expansion, and NK cell priming (9-11). As a damper to IL-18, IL-18 BPa blocks T cell infiltration into sites of inflammation, the production of pro-inflammatory cytokines by $CD8^+$ T cells and NK cells, and the expansion of Th17 responses (12-15), while it also encourages protective Th2-biased responses (16). IL-18 is elevated in the serum during chronic inflammation and kidney insufficiency (17-20), as well as in rheumatoid arthritis synovial fluid (21). IL-18 BP expression is upregulated in response to inflammatory stimulation of vascular endothelial cells, macrophages, microglia, and synovial fibroblasts (15, 21, 22). It circulates at elevated levels in systemic lupus erythematosus (SLE), active Crohn's disease, ulcerative colitis, alcoholic hepatitis, and chronic renal insufficiency (17-20, 23, 24), but it is lower in the synovial fluid of rheumatoid arthritis relative to osteoarthritis (21).

The anti-inflammatory effects of IL-18 BPa are observed *in vivo* as reduced development or severity of disease in experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA), contact hypersensitivity, LPS-induced shock, and a mouse model of hemophagocytic lymphohistiocytosis (HLH) (7, 12-16). IL-18 BP prevents the development and progression of atherosclerosis by reducing arterial lipid deposition, immune cell infiltration, and plaque instability (25, 26). Following ischemia/reperfusion injury in the heart, IL-18 BP limits inflammation and improves cardiac function (27). In cancer, IL-18 BP inhibits tumor progression and metastasis (28, 29).

The Quantikine™ Human IL-18 BPa Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human IL-18 BPa in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant human IL-18 BPa and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-18 BPa showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-18 BPa.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-18 BPa has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-18 BPa present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-18 BPa 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-18 BPa 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.
- Samples must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-18 BPa Microplate	894415	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-18 BPa.	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-18 BPa Conjugate	894416	21 mL of a polyclonal antibody specific for human IL-18 BPa conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-18 BPa Standard	894417	Recombinant human IL-18 BPa in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-73	895541	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-18	895335	21 mL of a buffered protein base with preservatives. *For cell culture supernate/urine samples.*	
Calibrator Diluent RD6-68	896002	21 mL of a animal serum with preservatives. *For serum/plasma samples.*	
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	895032	6 mL of 2 N sulfuric 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
- Test tubes for dilution of standards and samples
- Human IL-18 BPa Controls (optional; R&D Systems®, Catalog # QC27)

PRECAUTIONS

Calibrator Diluent RD6-68 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

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

Serum - Use a 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.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD6-68.

Urine samples require a 20-fold dilution. A suggested 20-fold dilution is 25 µL of sample + 475 µL of Calibrator Diluent RD5-18.

REAGENT PREPARATION

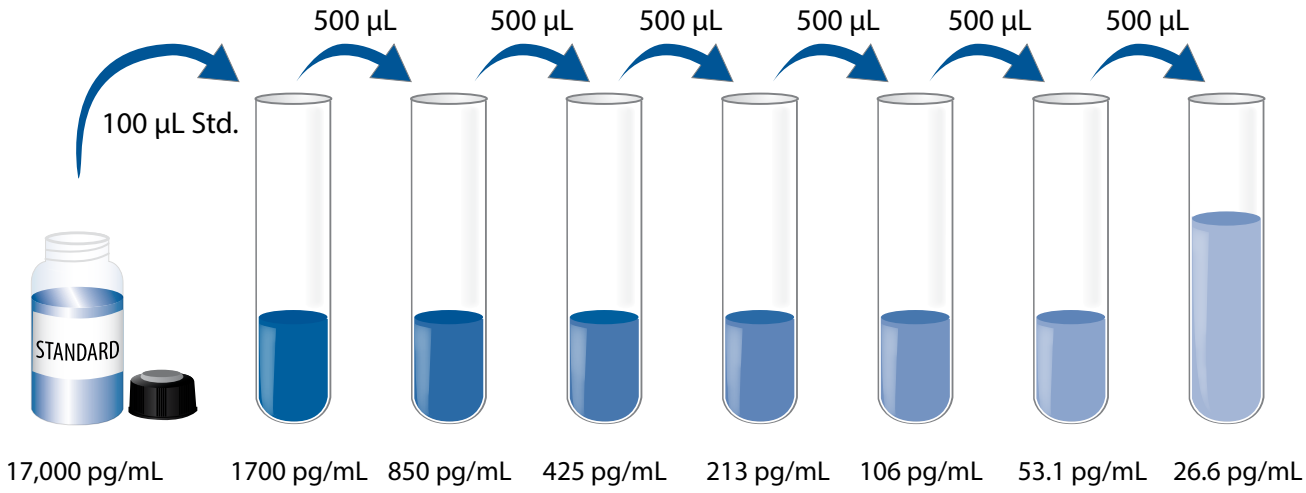
Bring all reagents to room temperature before use.

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 480 mL of 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.

Human IL-18 BPa Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-18 BPa Standard with deionized or distilled water. This reconstitution produces a stock solution of 17,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 20 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-18 (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6-68 (*for serum/plasma samples*) into the 1700 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 1700 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, controls, and samples be assayed in duplicate.

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 RD1-73 to each well.
4. Add 50 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.

   Note: *Pipette standard, controls, and samples within 15 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-18 BPa 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/Urine Samples: Incubate for 20 minutes at room temperature on the benchtop.
   For Serum/Plasma Samples: Incubate for 30 minutes at room temperature on the benchtop.
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.

*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.).

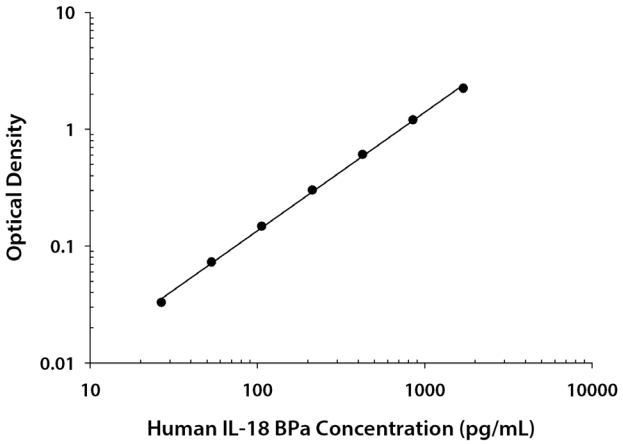
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-18 BPa concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

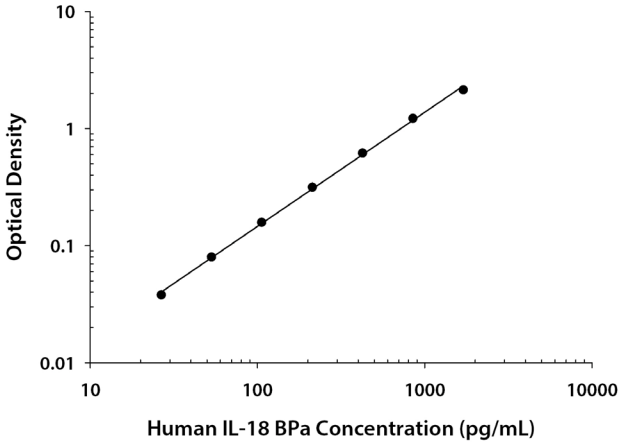
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.014	0.014	—
26.6	0.046 0.047	0.047	0.033
53.1	0.085 0.088	0.087	0.073
106	0.161 0.163	0.162	0.148
213	0.314 0.317	0.316	0.302
425	0.598 0.644	0.621	0.607
850	1.157 1.281	1.219	1.205
1700	2.108 2.400	2.254	2.240

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.014 0.016	0.015	—
26.6	0.052 0.054	0.053	0.038
53.1	0.094 0.095	0.095	0.080
106	0.168 0.177	0.173	0.158
213	0.313 0.346	0.330	0.315
425	0.602 0.661	0.632	0.617
850	1.230 1.243	1.237	1.222
1700	2.047 2.268	2.158	2.143

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/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	123	404	789	136	434	841
Standard deviation	5.50	9.49	21.1	9.47	32.8	59.1
CV (%)	4.5	2.3	2.7	7.0	7.6	7.0

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	109	368	740	115	362	692
Standard deviation	5.73	16.4	15.8	13.5	29.1	48.3
CV (%)	5.3	4.5	2.1	11.7	8.0	7.0

RECOVERY

The recovery of human IL-18 BPa spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	98-114%
Serum* (n=4)	102	92-113%
EDTA plasma* (n=4)	106	93-113%
Heparin plasma* (n=4)	105	92-119%

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human IL-18 BPa were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	105	104	99	105	98
	Range (%)	100-110	94-113	97-106	95-114	94-100
1:4	Average % of Expected	99	99	98	98	95
	Range (%)	92-107	85-111	92-106	86-105	92-101
1:8	Average % of Expected	95	88	92	94	89
	Range (%)	89-100	81-99	87-97	86-99	84-91
1:16	Average % of Expected	91	87	91	91	86
	Range (%)	85-96	80-93	87-96	89-96	78-91

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

SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of human IL-18 BPa ranged from 0.539-7.52 pg/mL. The mean MDD was 2.25 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human IL-18 BPa manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human IL-18 BPa 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)	3950	1910-7780	1350
EDTA plasma (n=35)	3878	2102-6718	1224
Heparin plasma (n=35)	3609	1778-6428	1195
Urine (n=18)	12,939	1836-29,073	7632

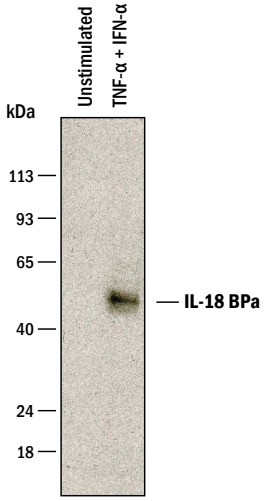
Cell Culture Supernates:

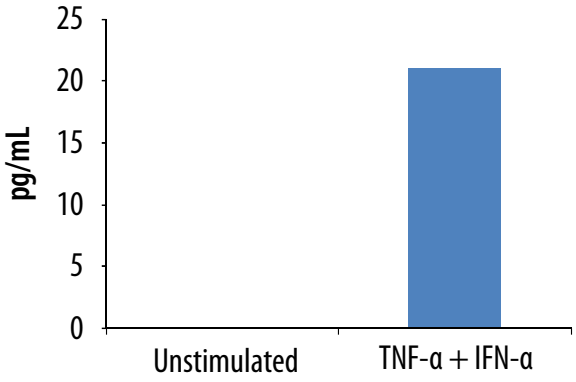
PBLs were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for human IL-18 BPa.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	ND	27.0
Stimulated	311	272

ND-Non-detectable

THP-1 human acute monocytic leukemia cells were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human IL-18 BPa, and measured 37.0 pg/mL.





Conditioned media from unstimulated or TNF-α + IFN-α stimulated HUVEC cells was resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with the detection antibody used in this kit. The number of picograms loaded per lane is based on the ELISA value for these samples. The unstimulated conditioned media was non-detectable for human IL-18 BPa and the stimulated measured 21 pg/mL in the Human IL-18 BPa Quantikine™ ELISA.

SPECIFICITY

This assay recognizes natural and recombinant human IL-18 BPa.

The factors listed below were prepared at 17 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 17 ng/mL in a mid-range recombinant human IL-18 BPa control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CEBP β
IL-1β
IL-1 RAcP
IL-1 RAPL1
IL-1 RAPL2
IL-1 Rrp2
IL-18 Rα
IL-18 Rβ
IL-33
IL-36α
IL-36β
IL-36γ
IL-36 Ra
IL-37
IL-37b
IL-38
IRF1
SIGIRR
ST2

Recombinant mouse:

CEBP β
IL-18
IL-18 BPc
IL-18 BPd
IL-18 R
IL-33
IL-36α
IL-36β
IL-36γ
IL-36 Ra
IRF1
SIGIRR
ST2

Recombinant rat:

IL-1α
IL-1ra
IL-18

Recombinant porcine:

IL-1α
IL-1ra

Other recombinants:

cotton rat IL-1α
equine IL-1ra
canine IL-18
feline IL-18

Recombinant human, porcine, and rhesus macaque IL-18 interfere at concentrations > 1.7 ng/mL.

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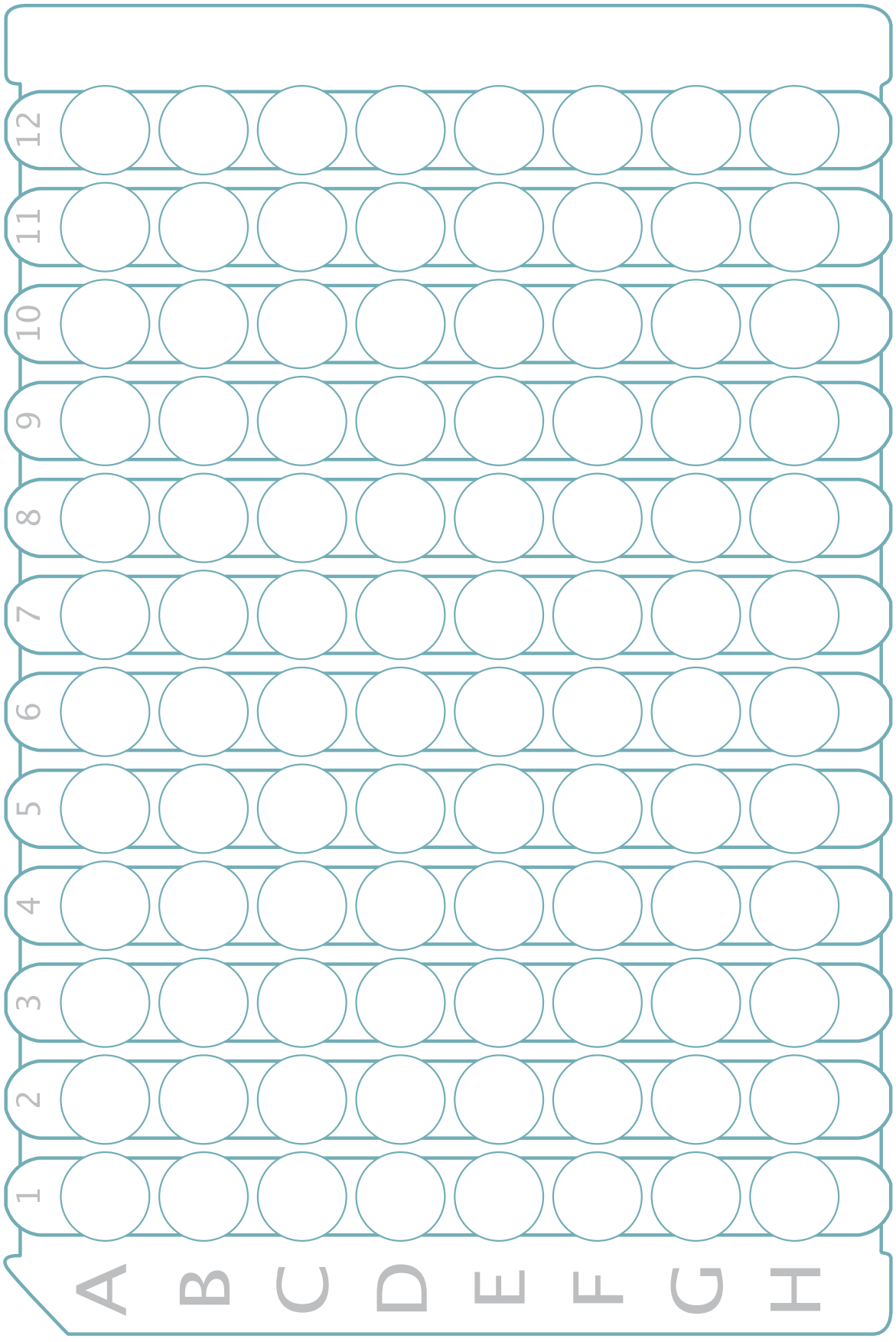
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES