Quantikine® ELISA

Human Total IL-18/IL-1F4 Immunoassay

Catalog Number DL180

For the quantitative determination of human free and complexed Interleukin 18 (Total IL-18) concentrations in cell culture supernates, serum, plasma, saliva, 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.

TABLE OF CONTENTS

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INTRODUCTION

Interleukin-18 (IL-18) is a proinflammatory cytokine in the IL-1 family that exerts distinct immune effects depending on the local cytokine environment. It is expressed as a 24 kDa precursor by endothelial and epithelial cells, keratinocytes, γδT cells, and phagocytes. The precursor is activated intracellularly by Caspase-1 mediated proteolysis to release the 17 kDa mature cytokine. The precursor can also be released by necrotic cells for extracellular cleavage by multiple proteases. IL-18 activation is induced by infection or tissue damage and contributes to disease pathology in chronic inflammation (1-3). IL-18 binds to the widely expressed IL-18 Rα which recruits IL-18 Rβ to form the signaling receptor complex (4, 5). Its bioactivity is negatively regulated by interactions with IL-18 binding proteins and virally encoded IL-18BP homologs (6). In the presence of IL-12 or IL-15, IL-18 enhances anti-viral Th1 immune responses by inducing IFN-γ production and the cytolytic activity of $CD8^+$ T cells and NK cells (7, 8). In the absence of IL-12 or IL-15, however, IL-18 promotes production of the Th2 cytokines IL-4 and IL-13 by $CD4^+$ T cells and basophils (9, 10). In the presence of IL-1β or IL-23, IL-18 induces the antigen-independent production of IL-17 by γδ T cells and $CD4^+$ T cells (11). IL-18 also promotes myeloid dendritic cell maturation and triggers neutrophil respiratory burst (12, 13). In cancer, IL-18 exhibits diverse activities including enhancing anti-tumor immunity, inhibiting or promoting angiogenesis, and promoting tumor cell metastasis (14). Mature human IL-18 shares approximately 63% amino acid sequence identity with mouse and rat IL-18 (15). Alternative splicing in human ovarian cancer generates an isoform that is resistant to Caspase-1 activation (16). A cell surface form can be expressed on M-CSF induced macrophages and released in response to bacterial endotoxin (17).

The Quantikine® Human Total IL-18/IL-1F4 Immunoassay is a 4.0 hour solid-phase ELISA designed to measure human IL-18 in cell culture supernates, serum, plasma, saliva, and urine. It contains *E. coli*-expressed recombinant human IL-18 and has been shown to accurately quantitate the recombinant factor free and in complex with IL-18 BPa. Results obtained using natural human IL-18 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.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Total IL-18 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-18 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for Total IL-18 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-18 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.
- If samples generate values higher than the highest standard, further 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.
- 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 Total IL-18 Microplate	898526	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Total IL-18.	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 Total IL-18 Standard	898529	2 vials of recombinant human IL-18 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Human Total IL-18 Conjugate	898527	21 mL of monoclonal antibody specific for Total IL-18 conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	12 mL of a buffered protein base with preservatives. *For serum/plasma/saliva samples.*	
Assay Diluent RD1-43	895521	11 mL of a buffered protein base with preservatives. *For cell culture supernate/urine samples. May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5P	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Streptavidin-HRP	898528	21 mL of a solution with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) 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.
- `100 mL and 1000 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Total IL-18 Controls (optional; R&D Systems®, Catalog # QC231).

PRECAUTIONS

IL-18 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.*
Grossly hemolyzed 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.

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

SAMPLE PREPARATION

Serum and plasma samples require a 3-fold dilution due to a matrix effect. A suggested 3-fold dilution is 50 µL of sample + 100 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Saliva samples require a 2-fold dilution due to a matrix effect. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *IL-18 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

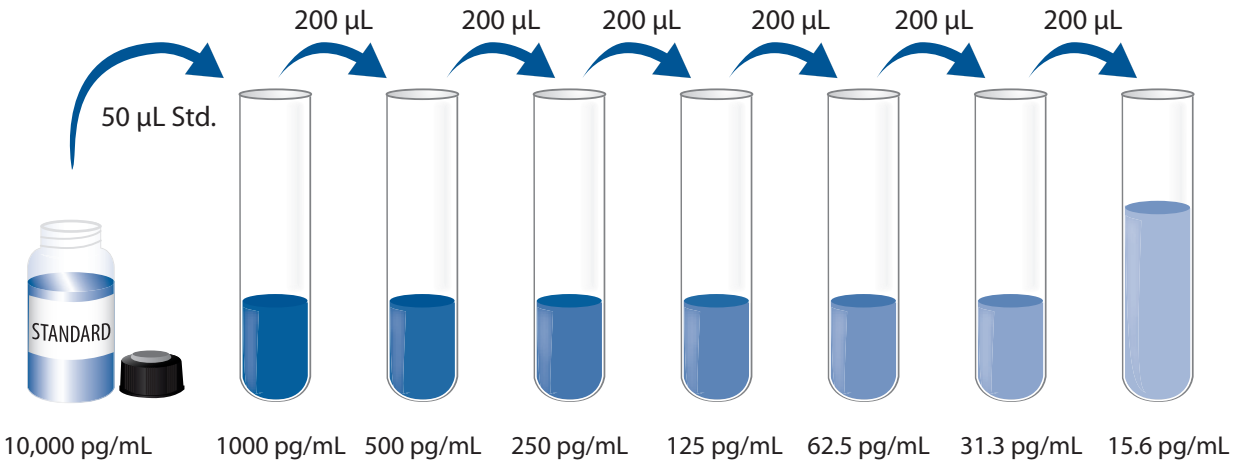
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Human Total IL-18 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Total IL-18 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 1000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, controls, and standards be assayed in duplicate.

Note: *IL-18 is 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 50 µL of Assay Diluent RD1-43 (*for cell culture supernate/urine samples*) or Assay Diluent RD1N (*for serum/plasma/saliva samples*) 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 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 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 Total IL-18 Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Streptavidin-HRP to each well. Cover with a new adhesive strip. Incubate for **30 minutes** at room temperature on the shaker.
9. Repeat the aspiration/wash as in step 5.
10. 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/Saliva Samples: Incubate for **30 minutes** at room temperature **on the benchtop.**
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.).

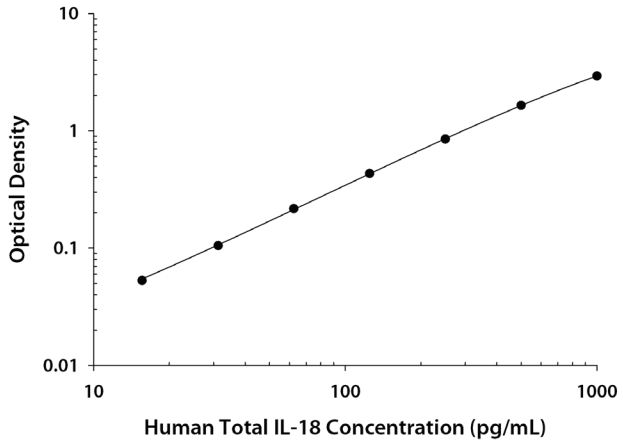
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Total IL-18 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

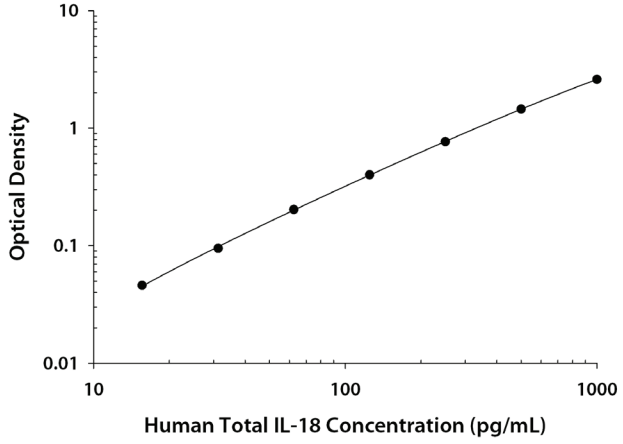
This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.012 0.013	0.013	—
15.6	0.064 0.067	0.066	0.053
31.3	0.116 0.119	0.118	0.105
62.5	0.220 0.240	0.230	0.217
125	0.440 0.451	0.446	0.433
250	0.858 0.870	0.864	0.851
500	1.657 1.663	1.660	1.647
1000	2.918 2.983	2.951	2.938

SERUM/PLASMA/SALIVA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.009 0.012	0.011	—
15.6	0.056 0.057	0.057	0.046
31.3	0.105 0.106	0.106	0.095
62.5	0.213 0.214	0.214	0.203
125	0.399 0.425	0.412	0.401
250	0.763 0.795	0.779	0.768
500	1.434 1.491	1.463	1.452
1000	2.596 2.632	2.614	2.603

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)	175	301	636	197	381	739
Standard deviation	5.49	8.06	24.6	27.4	46.7	81.1
CV (%)	3.1	2.7	3.9	13.9	12.3	11.0

SERUM/PLASMA/SALIVA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	126	251	495	126	248	494
Standard deviation	3.85	6.33	14.6	10.9	19.7	41.6
CV (%)	3.1	2.5	2.9	8.7	7.9	8.4

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	90-102%
Serum* (n=4)	100	96-105%
EDTA plasma* (n=4)	104	93-113%
Heparin plasma* (n=4)	102	88-110%
Saliva* (n=4)	108	94-120%
Urine (n=4)	91	84-100%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of natural human Total IL-18 were serially 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)	Saliva* (n=4)	Urine* (n=4)
1:2	Average % of Expected	105	97	95	96	100	105
	Range (%)	100-110	96-99	93-96	94-99	96-104	103-107
1:4	Average % of Expected	107	96	93	96	100	106
	Range (%)	102-113	93-97	88-96	93-99	98-103	101-110
1:8	Average % of Expected	109	97	94	95	101	106
	Range (%)	102-113	92-101	88-98	91-98	97-106	100-114
1:16	Average % of Expected	112	100	95	98	99	104
	Range (%)	105-115	97-106	89-101	94-100	95-104	96-113

*Samples were diluted prior to assay.

SENSITIVITY

Fifty-two assays were evaluated and the minimum detectable dose (MDD) of human Total IL-18 ranged from 0.296-5.15 pg/mL. The mean MDD was 1.20 pg/mL for RD1-43 (*cell culture supernate/urine assay*) and 1.25 pg/mL for RD1N (*serum/plasma/saliva assay*).

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-18 manufactured at R&D Systems®.

The NIBSC/WHO Human IL-18 Reference Reagent 03/200 was evaluated in this kit. The dose response curve of the reference reagent 03/200 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human Total IL-18/IL-1F4 kit to approximate NIBSC/WHO 03/200 Units, use the equations below.

Cell Culture Supernate/Urine Assay: NIBSC/WHO (03/200) approximate value (IU/mL) = 0.014 x Quantikine® Human Total IL-18/IL-1F4 value (pg/mL)

Serum/Plasma/Saliva Assay: NIBSC/WHO (03/200) approximate value (IU/mL) = 0.011 x Quantikine® Human Total IL-18/IL-1F4 value (pg/mL)

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

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Total IL-18 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=30)	183	84.8-358	69.7
EDTA plasma (n=30)	183	81.5-344	68.1
Heparin plasma (n=30)	182	87.5-333	65.8

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Saliva (n=10)	57.1	70	ND-69.8
Urine (n=10)	44.0	30	ND-74.0

ND=Non-detectable

Cell Culture Supernates - THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1 µg/mL LPS for 5 hours followed by 20 µM Nigericin for 1 hour. Aliquots of the cell culture supernates were removed and assayed for levels of human Total IL-18.

Condition	(pg/mL)
Unstimulated	19.6
Stimulated	562

SPECIFICITY

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

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 recombinant human Total IL-18 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IFN-β
IFN-γ
IL-1α
IL-1β
IL-10
IL-11
IL-12
IL-13
IL-15
IL-16
IL-17
IL-18 BPa
IL-18 R
IL-19

Recombinant mouse:

IL-18

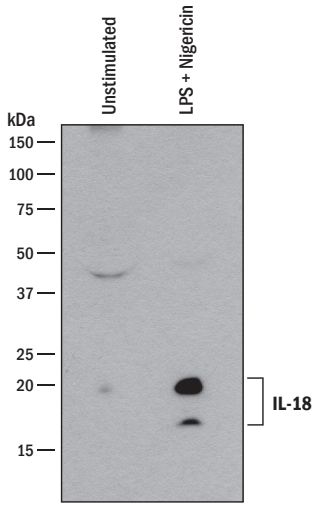
Recombinant rat:

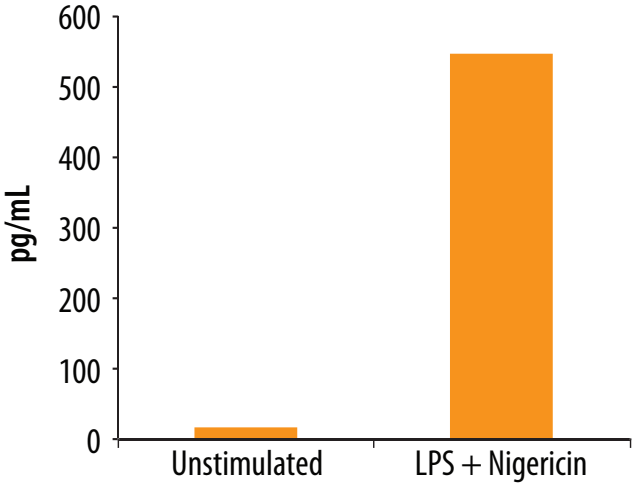
IL-18

Recombinant human Pro-IL-18 cross-reacts approximately 0.06% in this assay.

Recombinant human IL-18/IL-18 BPa Complex cross-reacts approximately 24.5% in this assay.

Recombinant rhesus macaque IL-18 (aa 37-193) cross-reacts approximately 41% in this assay.





Conditioned media samples from THP-1 human acute monocytic leukemia cells were analyzed by Western Blot and Quantikine® ELISA. The cells were unstimulated or stimulated with 1 µg/mL LPS for 5 hours followed by 20 µM Nigericin for 1 hour prior to media harvest. Conditioned media samples were resolved under reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with a goat anti-human IL-18 antibody. The band intensity from the Western Blot correlates with the Quantikine® ELISA sample value. The upper band size is consistent with the human Pro-IL-18, while the lower band is consistent with the mature human IL-18.

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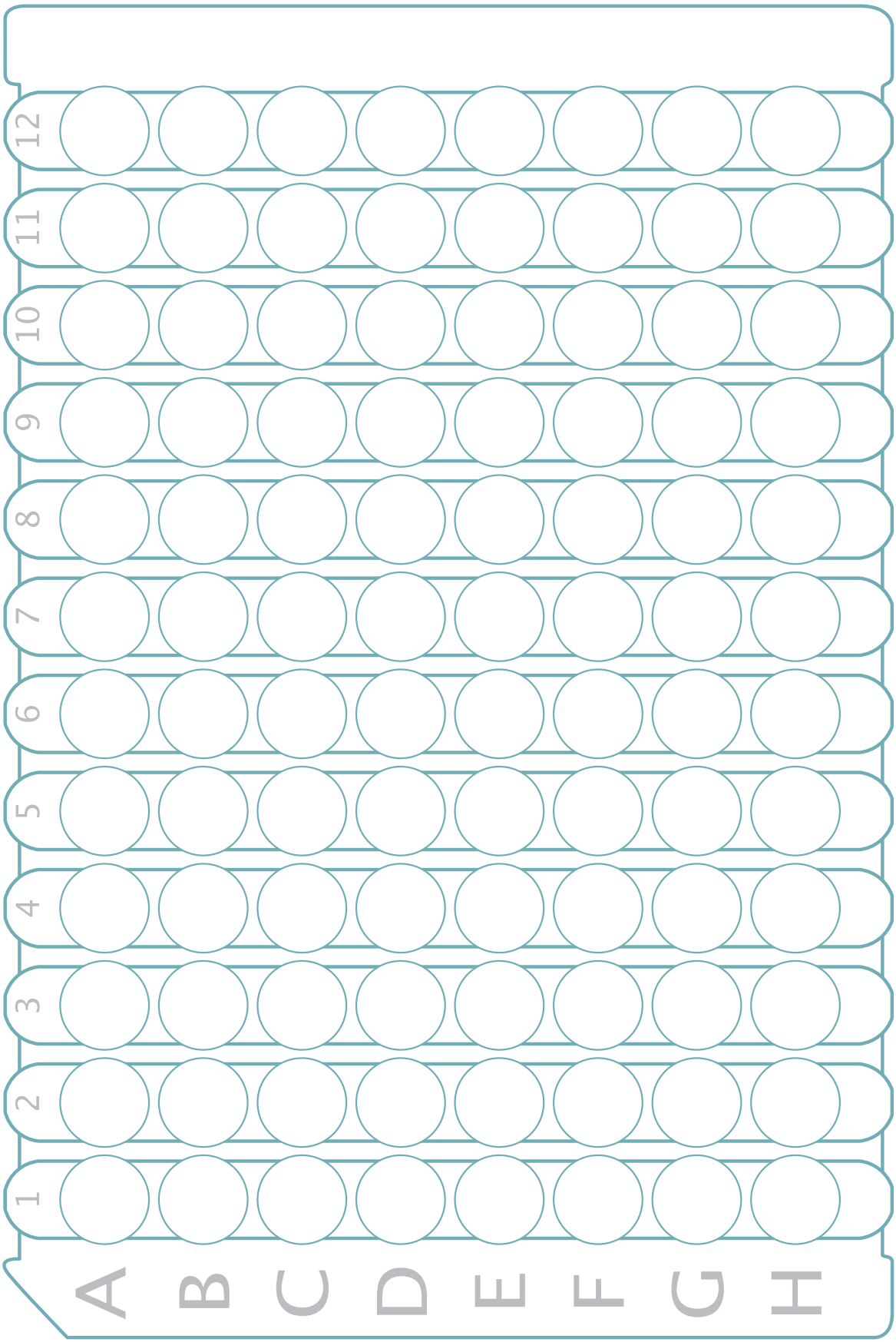
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES