

Quantikine[®]

Human IL-19 Immunoassay

Catalog Number D1900

For the quantitative determination of human Interleukin 19 (IL-19) concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

This package insert must be read in its entirety before using this product.

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**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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INTRODUCTION

Interleukin 19 (IL-19) is a member of the IL-10 family of class II α -helical cytokines that contains viral and cellular homolog proteins (1 - 3). Mature human IL-19 is secreted as a 35 - 40 kDa N-glycosylated monomer (3, 4). It shares 69% amino acid (aa) sequence identity with mouse and rat IL-19. Under normal conditions, IL-19 expression is primarily restricted to monocytes. Upon inflammatory stimulation, it is upregulated in monocytes as well as in keratinocytes, smooth muscle cells, and airway epithelial cells (3, 5 - 8). IL-19 induces the release of inflammatory mediators and KGF from monocytes, dendritic cells, lung epithelial cells, hepatocytes, or CD8⁺ T cells (9 - 12). It drives T-helper cell differentiation towards a Th2 response, inducing both IL-10 and additional production of itself (10, 13, 14).

IL-19 exerts its effects through a heterodimeric complex composed of the transmembrane class II cytokine receptors IL-20 R α and IL-20 R β (1, 15, 16). It binds to IL-20 R β which then associates with IL-20 R α to form a signaling receptor complex (17, 18). The two receptor subunits do not interact in the absence of ligand (17). This receptor complex also mediates IL-20 and IL-24 effects (15 - 19). In addition, IL-20 R α heterodimerizes with IL-10 R β to form the functional receptor complex for IL-26, and IL-20 R β heterodimerizes with IL-22 R to form the functional receptor complex for IL-20 and IL-24 (15, 18, 20). IL-20 R α is expressed in skin, heart, placenta, salivary gland, testis, and prostate gland (15, 16). Strong IL-20 R β expression is normally restricted to skin, testis, and prostate (15, 16). The expression of both IL-20 R α and IL-20 R β are upregulated in psoriatic skin lesions on keratinocytes, immune cells, and endothelial cells (16).

Serum IL-19 is elevated in asthma, uremia, and septic shock (11, 13, 21). It is produced within sites of inflammation such as airway epithelia in asthma, basal and suprabasal keratinocytes in psoriasis, and vascular smooth muscle cells following arterial injury (5, 7, 12, 22). IL-19 plays a protective role following balloon angioplasty but promotes neutrophil infiltration and tissue damage in mouse models of septic shock (7, 11).

The Quantikine Human IL-19 immunoassay is a 4.5 hour solid phase ELISA designed to measure IL-19 in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains NS0-expressed recombinant human IL-19 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-19 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Human IL-19 immunoassay can be used to determine relative mass values for naturally occurring human IL-19.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-19 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-19 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for IL-19 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-19 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, 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.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

IL-19 Microplate (Part 892688) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against IL-19.

IL-19 Conjugate (Part 892689) - 21.5 mL of a mouse monoclonal antibody against IL-19 conjugated to horseradish peroxidase with preservatives.

IL-19 Standard (Part 892690) - 20 ng of recombinant human IL-19 in a buffered protein solution with preservatives; lyophilized.

Assay Diluent RD1-19 (Part 895467) - 11 mL of a buffered protein solution with preservatives.

Calibrator Diluent RD5K (Part 895119) - 21 mL of a buffered protein solution with preservative.

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of a buffered surfactant with preservatives.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895032) - 6 mL of a 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-19	
	Calibrator Diluent RD5K	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	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.*

*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.
- Human IL-19 Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

IL-19 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ 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 $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated 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 and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: *Saliva values are decreased when a Salivette™ or other collection device is used.*

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 $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles. For more information on collecting and handling urine specimens, refer to the National Committee for Clinical Laboratory Standards (NCCLS) publication GP16-T.

Human Milk - Centrifuge for 15 minutes at 10,000 x g at 2 - 8° C. Collect the aqueous fraction and repeat this process twice more for a total of 3 times. Assay immediately or aliquot and store at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human milk samples require at least a 20-fold dilution. A suggested 20-fold dilution is 20 μ L of sample + 380 μ L of Calibrator Diluent RD5K.

Salivette™ is a trademark of Sarstedt, Inc.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

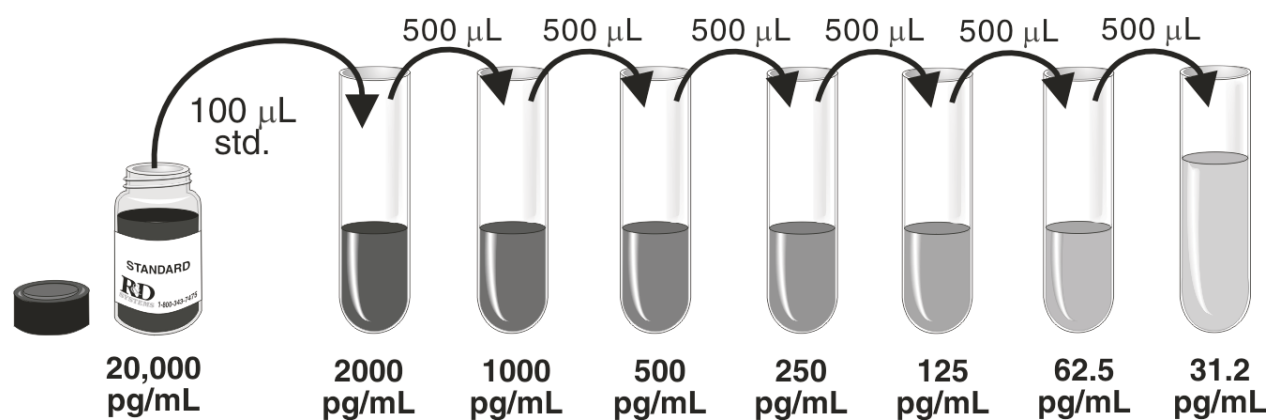
Note: High concentrations of IL-19 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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.

IL-19 Standard - Reconstitute the human IL-19 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a 10X stock solution of 20 ng/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 900 μL of Calibrator Diluent RD5K into the 2000 pg/mL tube and 500 μL of Calibrator Diluent RD5K 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. Calibrator Diluent RD5K 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: *High concentrations of IL-19 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 RD1-19 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.
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 IL-19 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. Incubate for 30 minutes at room temperature. **Protect from light.**
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.

*Human milk samples require dilution. See the Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and Standards as instructed.



2. Add 100 μL Assay Diluent RD1-19 to each well.



3. Add 50 μL Standard, control, or sample* to each well. Incubate 2 hours at RT.



4. Aspirate and wash 4 times.



5. Add 200 μL Conjugate to each well. Incubate 2 hours at RT.



6. Aspirate and wash 4 times.



7. Add 200 μL Substrate Solution to each well. Incubate 30 minutes at RT.

Protect from light.



8. Add 50 μL Stop Solution to each well. Read at 450 nm within 30 minutes.
 λ correction 540 or 570 nm

*Human milk samples 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.

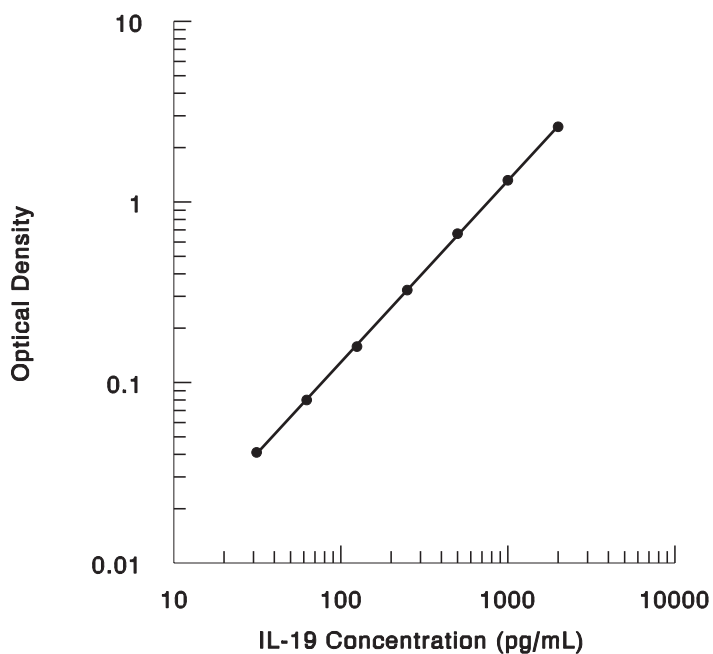
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the IL-19 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding IL-19 concentration.

If the 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	0.014 0.015 0.055	0.015	—
31.2	0.056 0.092	0.056	0.041
62.5	0.097 0.172	0.095	0.080
125	0.174 0.338	0.173	0.158
250	0.342 0.674	0.340	0.325
500	0.689 1.298	0.682	0.667
1000	1.370 2.611	1.334	1.319
2000	2.637	2.624	2.609

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.
- 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.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	292	805	1586	269	739	1478
Standard deviation	10.3	19.0	41.8	22.0	56.8	103
CV (%)	3.5	2.4	2.6	8.2	7.7	7.0

RECOVERY

The recovery of IL-19 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample	Average % Recovery	Range
Cell culture media (n=4)	98	93 - 104%
Serum (n=4)	93	87 - 97%
EDTA plasma (n=4)	94	85 - 102%
Heparin plasma (n=4)	93	87 - 99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of IL-19 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates* (n=5)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva* (n=5)	Human milk* (n=5)
1:2	Average % of Expected	102	100	100	98	103	99
	Range (%)	98 - 107	95 - 104	95 - 102	92 - 108	99 - 108	94 - 104
1:4	Average % of Expected	103	104	103	101	101	99
	Range (%)	97 - 110	99 - 108	98 - 108	95 - 112	95 - 109	95 - 102
1:8	Average % of Expected	101	105	105	102	99	97
	Range (%)	96 - 107	97 - 114	97 - 109	95 - 116	91 - 106	92 - 100
1:16	Average % of Expected	99	102	100	98	94	93
	Range (%)	94 - 101	98 - 106	94 - 107	92 - 110	90 - 103	86 - 97

*Samples were diluted prior to assay.

SENSITIVITY

Fifty-six assays were evaluated and the minimum detectable dose (MDD) of IL-19 ranged from 2.2 - 12.2 pg/mL. The mean MDD was 5.4 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 NS0-expressed recombinant human IL-19 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Eighty-one samples drawn from apparently healthy volunteers were evaluated for the presence of IL-19 in this assay. Only two samples measured above the low standard, 31.2 pg/mL. No medical histories were available for the donors used in this study.

Sample Type	Sample 1 (pg/mL)	Sample 2 (pg/mL)
Serum (n=81)	49.3	81.9
EDTA plasma (n=81)	49.7	83.9
Heparin plasma (n=81)	52.3	90.5

Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of IL-19 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)
Saliva* (n=19)	935	84%	ND - 2954
Urine (n=18)	130	11%	ND - 195

*Samples may have been diluted prior to assay.

ND = Non-detectable

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Human milk** (n=5)	115	25.3 - 177	64.0

**Samples were diluted prior to assay.

Cell Culture Supernates -

Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal calf serum, 5 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 μ g/mL PHA for the time indicated in the table below. Aliquots of the cell culture supernates were removed and assayed for levels of natural IL-19.

Condition	Observed Levels (pg/mL)
Unstimulated, Day 1	216
Unstimulated, Day 6	ND
Stimulated, Day 1	235
Stimulated, Day 6	347

ND = Non-detectable

Human monocyte cells were cultured in RPMI supplemented with 10% fetal calf serum, 25 ng/mL recombinant human GM-CSF, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were cultured stimulated with 50 ng/mL LPS for the final 24 hours. An aliquot of the cell culture supernate was removed, assayed for levels of natural IL-19, and measured 5084 pg/mL.

SPECIFICITY

This assay recognizes recombinant and natural human IL-19. 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 IL-19 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-10
IL-10 R α
IL-10 R β
IL-20
IL-20 R α
IL-20 R β
IL-22
IL-22 R
IL-24
IL-26

Recombinant mouse:

IL-10
IL-10 R α
IL-10 R β
IL-19
IL-20
IL-20 R α
IL-20 R β
IL-22 R
IL-24

Other recombinants:

canine IL-10
equine IL-10
feline IL-10
guinea pig IL-10
viral CMV IL-10
rat IL-10
rat IL-22

REFERENCES

1. Pestka, S. *et al.* (2004) *Annu. Rev. Immunol.* **22**:929.
2. Zdanov, A. (2004) *Curr. Pharm. Des.* **10**:3873.
3. Gallagher, G. *et al.* (2000) *Genes Immun.* **1**:442.
4. Chang, C. *et al.* (2003) *J. Biol. Chem.* **278**:3308.
5. Huang, F. *et al.* (2008) *J. Allergy Clin. Immunol.* **121**:1415.
6. Wolk, K. *et al.* (2002) *J. Immunol.* **168**:5397.
7. Tian, Y. *et al.* (2008) *Am. J. Pathol.* **173**:901.
8. Hsing, C-H. *et al.* (2006) *Ann. Thorac. Surg.* **81**:2196.
9. Laio, Y-C. *et al.* (2002) *J. Immunol.* **169**:4288.
10. Jordan, W.J. *et al.* (2005) *Eur. J. Immunol.* **35**:1576.
11. Hsing, C-H. *et al.* (2008) *Shock* **29**:7.
12. Li, H-H. *et al.* (2005) *Br. J. Dermatol.* **153**:591.
13. Laio, S-C. *et al.* (2004) *J. Immunol.* **173**:6712.
14. Gallagher, G. *et al.* (2004) *Int. Immunopathol.* **4**:615.
15. Parrish-Novak, J. *et al.* (2002) *J. Biol. Chem.* **277**:47517.
16. Blumberg, H. *et al.* (2001) *Cell* **104**:9.
17. Pletnev, S. *et al.* (2003) *Biochemistry* **42**:12617.
18. Dumoutier, L. *et al.* (2001) *J. Immunol.* **167**:3545.
19. Wang, M. *et al.* (2002) *J. Biol. Chem.* **277**:7341.
20. Sheikh, F. *et al.* (2004) *J. Immunol.* **172**:2006.
21. Hsing, C-H. *et al.* (2007) *Nephrol. Dial. Transplant.* **22**:2230.
22. Romer, J. *et al.* (2003) *J. Invest. Dermatol.* **121**:1306.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
2									
1									
	A	B	C	D	E	F	G	H	