Quantikine™ QuicKit™ ELISA

Human IL-17 Immunoassay

Catalog Number QK317

For the quantitative determination of human Interleukin 17 (IL-17) 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

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	3
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	8
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

Manufactured and Distributed by:

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

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373   (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Human Interleukin 17 (IL-17), also known as IL-17A and CTLA-8, is a 15-20 kDa, variably glycosylated polypeptide that belongs to the IL-17 family of cytokines (1-5). Its alternate name, CTLA-8, originated from rodent studies where an activated hybridoma was created from the fusion of a mouse cytotoxic and a rat T cell lymphoma cell line. The molecule of interest in this study was assumed to have come from the mouse cytotoxic lymphocyte cell (thus the CTL designation), whereas, in fact, it was a rat lymphocyte molecule. Human IL-17/17A is synthesized as a 155 amino acid (aa) precursor that contains a 23 aa signal sequence and a 133 aa mature region that possesses a cysteine-knot fold (4-6). In both human and mouse, there is one conserved N-linked glycosylation site that likely contributes 5 kDa to its native molecular weight. IL-17A forms both a 32-38 kDa disulfide-linked homodimer, and a 40-45 kDa covalent heterodimer with IL-17F (7-9). Most secreted IL-17A is in the form of the IL-17A:F heterodimer, however, the IL-17A:A homodimer is the most bioactive of the two forms (8). Mature human IL-17A is 61%, 74%, and 99% aa identical to mouse, porcine, and chimpanzee IL-17A, respectively (10-12). Mammalian cells known to produce IL-17 are the $CD4^+$ Th17 T cells, Paneth cells, $GR1^+CD11b^+$ myeloid suppressor cells, $CD27^-$γδ T cells, $CD1^+NK1.1^-$iNKT cells, and $CD3^-CD4^+$ LTi-like cells (9, 13-17).

A high affinity receptor for human IL-17 has been reported, and appears to be a heteromultimer of IL-17RA and IL-17RC, likely in a 2:1 ratio (1, 18). IL-17RA is a 130 kDa, type I transmembrane glycoprotein that bears no resemblance to members of the cytokine, TNF or immunoglobulin receptor superfamily (2, 10, 15). IL-17RC is also a type I transmembrane protein, approximately 90-95 kDa in size, that shares less than 30% aa identity with IL-17RA (19, 20). Both receptors are needed for IL-17A and IL-17A:F activity. The two receptors appear to form a functional association following ligand binding to IL-17RA (1, 21, 22).

IL-17 is best known for its participation in the recruitment and survival of neutrophils (14, 15, 23, 24, 25). Its induction was initially described to be the result of antigen stimulation of dendritic cells, resulting in IL-23 secretion. In a T cell receptor-independent event, IL-23 induces T cell production of IL-17 (14). Once secreted, IL-17 in the bone marrow would seem to induce stromal/fibroblast expression of both G-CSF and stem cell factor (membrane form), an effect that increases polymorphonuclear neutrophils (PMN) differentiation and production. IL-17 may complement this by directly blocking neutrophil apoptosis, promoting greater circulating PMN numbers (23). In the tissues, IL-17 would also seem to promote neutrophil extravasation, principally through its effects on macrophages and endothelial cells (EC). On macrophages, IL-17 induces TNF-α, IL-1β and IL-6 production (26). TNF-α and IL-1β then act on local ECs to induce G-CSF secretion, an effect that is potentiated by IL-17 (27). IL-17 further contributes to PMN influx by inducing EC CXC chemokine release and NO production, which may increase vascular permeability (14, 28). IL-17 effects are not limited to inflammation. In synovial joints, IL-17 upregulates RANKL expression on osteoblasts. This provides a stimulus for osteoclast formation and subsequent bone resorption (24). In conjunction with IL-4 and CD40L, IL-17A also promotes the generation of IgE secreting cells (29). And in white fat, IL-17A inhibits adipocyte differentiation from preadipocytes, and impairs glucose uptake by mature adipocytes (30).

The Quantikine™ QuicKit Human IL-17 Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human IL-17 levels in cell culture supernates, serum, and plasma. It contains HEK293-expressed recombinant human IL-17 and antibodies raised against the recombinant protein. Results obtained using natural human IL-17 showed linear curves that were parallel to the standard curves obtained using the QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-17.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked polyclonal detection antibody, specific for human IL-17. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of IL-17 bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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-17 Standard	899104	2 vials of recombinant human IL-17 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IL-17 Capture Ab Concentrate	899102	Lyophilized tagged monoclonal antibody specific for human IL-17.	May be stored for up to 1 month at 2-8 °C.*
Human IL-17 Detection Ab Concentrate	899103	400 µL of a polyclonal antibody specific for human IL-17 conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-68	895528	11 mL/vial of a buffered protein base with blue dye and preservatives. *Use diluted 1:2 in this assay.*	
Calibrator Diluent RD5-68	896030	21 mL of a buffered protein base 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	895032	6 mL of 2N 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
- 50 mL and 500 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
- Human IL-17 Controls (optional; R&D Systems®, Catalog # QC272)

PRECAUTIONS

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.*

SAMPLE PREPARATION

Cell culture supernates, serum, and plasma can be tested neat. Some supernates may require dilution due to high endogenous levels.

Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Assay Diluent RD1-68 (diluted 1:2) - Add 5 mL of Assay Diluent RD1-68 to 5 mL of deionized or distilled water to prepare 10 mL of Assay Diluent RD1-68 (diluted 1:2).

Human IL-17 Capture Ab Concentrate - Refer to the vial label for reconstitution volume. Reconstitute the Human IL-17 Capture Ab Concentrate with Assay Diluent RD1-68 (diluted 1:2). This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

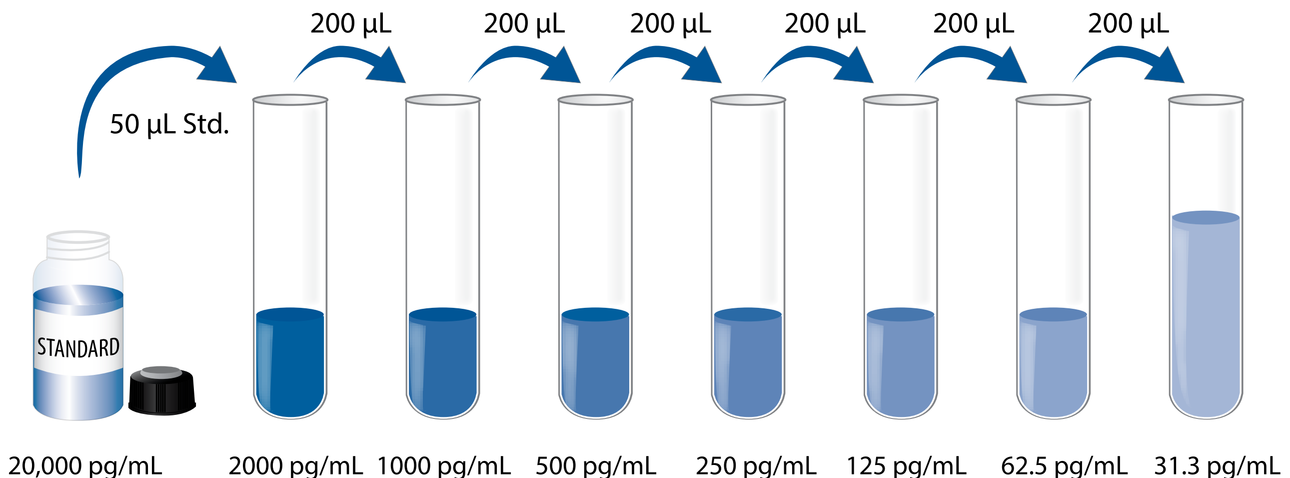
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-68 (diluted 1:2). For a full plate, add 300 µL of reconstituted Human IL-17 Capture Ab stock and 300 µL of Human IL-17 Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-68 (diluted 1:2).

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

Human IL-17 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human IL-17 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-68 into the 2000 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 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-68 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 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. 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.
8. 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.

For research use only. Not for use in diagnostic procedures.

CALCULATION OF RESULTS

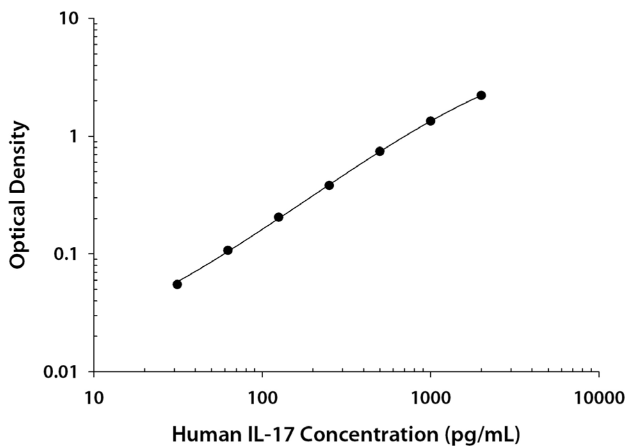
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-17 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	0.013 0.016	0.015	—
31.3	0.067 0.072	0.070	0.055
62.5	0.118 0.126	0.122	0.107
125	0.217 0.222	0.220	0.205
250	0.395 0.396	0.396	0.381
500	0.744 0.772	0.758	0.743
1000	1.355 1.357	1.356	1.341
2000	2.229 2.237	2.233	2.218

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	197	1025	201	1090
Standard deviation	7.09	56.4	6.41	55.2
CV (%)	3.6	5.5	3.2	5.1

RECOVERY

The recovery of human IL-17 spiked to three levels in samples throughout the range of the assay was evaluated. Samples were diluted prior to assay.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	112	106-118
Serum (n=2)	83	79-86
EDTA plasma (n=2)	115	107-122
Heparin plasma (n=2)	110	106-116

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-17 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=10)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)
1:2	Average % of Expected	90	90	95	90
	Range (%)	77-101	90-91	94-97	86-94
1:4	Average % of Expected	88	94	89	84
	Range (%)	80-98	89-98	86-93	82-86
1:8	Average % of Expected	90	103	85	86
	Range (%)	70-110	97-108	82-89	84-89
1:16	Average % of Expected	86	109	82	84
	Range (%)	75-101	105-112	78-86	82-87

For research use only. Not for use in diagnostic procedures.

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of human IL-17 ranged from 1.05-6.00 pg/mL. The mean MDD was 2.98 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 HEK293-expressed recombinant human IL-17 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Ten samples from apparently healthy volunteers were evaluated for the presence of human IL-17 in this assay. All samples measured less than the lowest standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates:
$CD4^+$ T cells were isolated from human PBMCs using the MagCellect™ Human $CD4^+$ T cell Isolation Kit (R&D Systems® Catalog # MAGH102). $CD4^+$ T cells were then seeded at 5×10^5/mL and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. T cells were left unstimulated in culture media, or cultured with immobilized Human CD3ε Antibody (R&D Systems, Catalog # MAB100) coated at 1 µg/mL and 5 µg/mL soluble Human CD28 Antibody (R&D Systems, Catalog # MAB342) for 5 days. Stimulated cells were then treated with 10 ng/mL PMA and 500 ng/mL Ionomycin calcium salt (Tocris™, Catalog # 1704) for 24 hours. An aliquot of cell culture supernate was removed and assayed for human IL-17.

Condition	(pg/mL)
Unstimulated	ND
Stimulated	244

ND=Non-Detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-17.

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 low level recombinant human IL-17 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:	Recombinant rat:
IL-17B	IL-17	IL-17A
IL-17C	IL-17A	IL-17F
IL-17D	IL-17B	
IL-17E	IL-17C	
IL-17F	IL-17D	
IL-17RB	IL-17E	
IL-17RD	IL-17F	
IL-17RE	IL-17RB	
	IL-17RD	
	IL-17RE	

Samples with abnormally high levels of Albumin interfere in this assay.

Recombinant human (rh) IL-17A/F Heterodimer interferes at levels > 1.56 ng/mL and cross-reacts approximately 6.5% in this assay.

Recombinant human IL-17RA and rhIL-17RC interfere at levels > 6.25 ng/mL and 1.56 ng/mL, respectively.

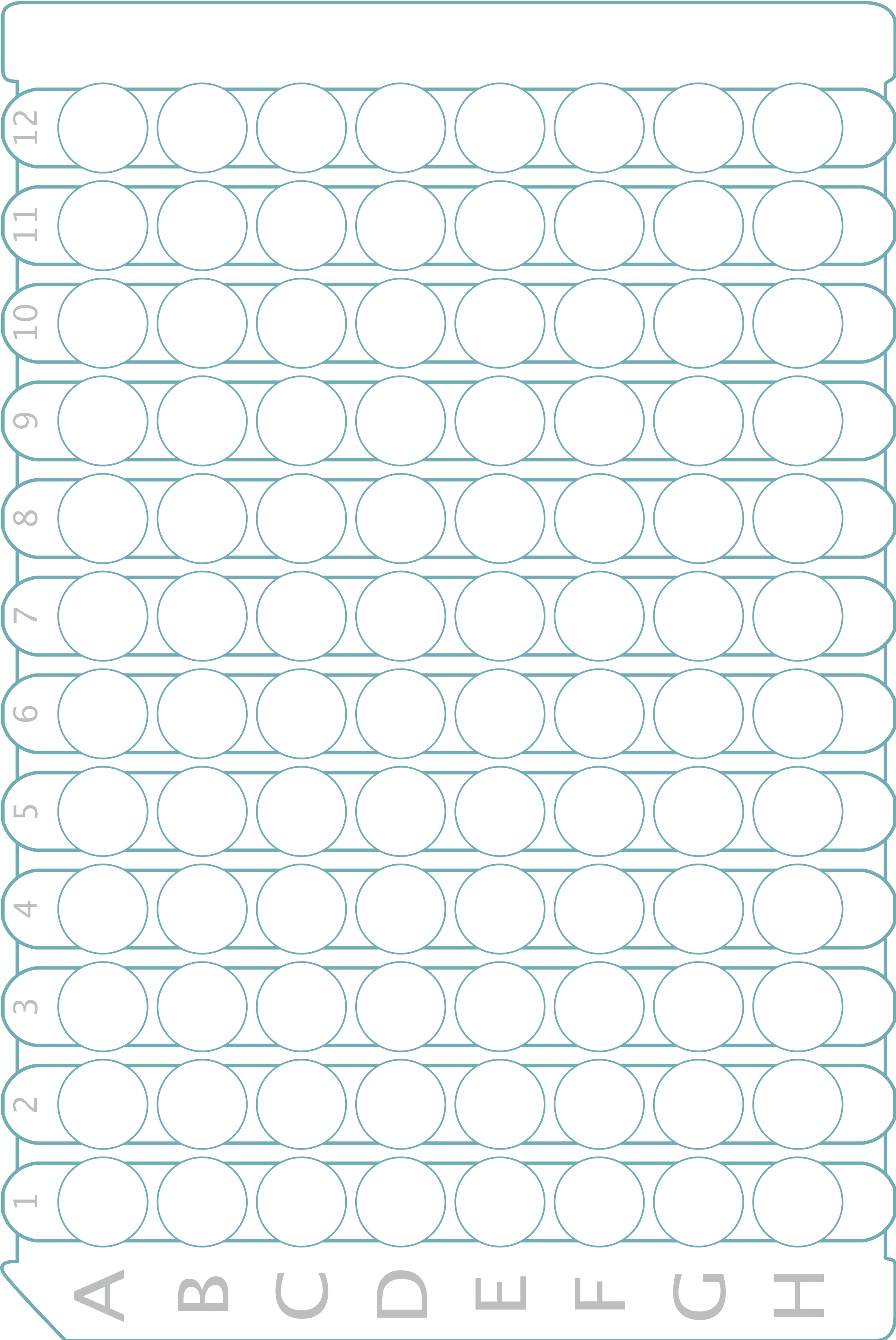
Recombinant human IL-17 R interferes at levels > 25 ng/mL.

REFERENCES

1. Gaffen, S. (2009) Nat. Rev. Immunol. **9**:556.
2. Iwakura, Y. *et al.* (2008) Immunol. Rev. **226**:57.
3. Zhang, X. *et al.* (2011) Protein Cell **2**:26.
4. Yao, Z. *et al.* (1995) J. Immunol. **155**:5483.
5. Fossiez, F. *et al.* (1996) J. Exp. Med. **183**:2593.
6. Hymowitz, S.G. *et al.* (2001) EMBO J. **20**:5332.
7. Chang, S. and C. Dong (2007) Cell Res. **17**:435.
8. Wright, J.F. *et al.* (2007) J. Biol. Chem. **282**:13447.
9. Liang, S.C. *et al.* (2007) J. Immunol. **179**:7791.
10. Yao, Z. *et al.* (2005) Immunity 3:811.
11. Katoh, S. *et al.* (2004) J. Interferon Cytokine Res. **24**:553.
12. GenBank Accession #:XP_527408.
13. Romagnani, S. *et al.* (2009) Mol. Immunol. **47**:3.
14. Kolls, J.K. and A. Linden (2004) Immunity **21**:467.
15. Witowski, J. *et al.* (2004) Cell. Mol. Life Sci. **61**:567.
16. Cua, D.J. and C.M. Tato (2010) Nat. Rev. Immunol. **10**:479..
17. Shin, H.C. *et al.* (1998) Cytokine **10**:841.
18. Yao, Z. *et al.* (1997) Cytokine **9**:794.
19. Haudenschild, D. *et al.* (2002) J. Biol. Chem. **277**:4309.
20. Toy, D. *et al.* (2006) J. Immunol. **177**:36.
21. Hu, Y. *et al.* (2010) J. Immunol. **184**:4307.
22. Ely, L.K. *et al.* (2009) Nat. Immunol. **10**:1245.
23. Schwarzenberger, P. *et al.* (2000) J. Immunol. **164**:4783.
24. Yu, J.J. and S.L. Gaffen (2008) Front. Biosci. **13**:170.
25. Khader, S.A. and R. Gopal (2010) Virulence **1**:423.
26. Jovanovic, D.V. *et al.* (1998) J. Immunol. **160**:3513.
27. Numasaki, M. *et al.* (2004) Immunol. Lett. **95**:97.
28. Miljkovic, D. *et al.* (2003) Cell. Mol. Life Sci. **60**:518.
29. Milovanovic, M. *et al.* (2010) J. Invest. Dermatol. **130**:2621.
30. Zuniga, L.A. *et al.* (2010) J. Immunol. **185**:6947.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



For research use only. Not for use in diagnostic procedures.

NOTES

NOTES

All trademarks and registered trademarks are the property of their respective owners.

©2021 R&D Systems®, Inc.

09.21 753148.0 9/21