

Quantikine[®]

Mouse IL-17 Immunoassay

Catalog Number M1700

SM1700

PM1700

For the quantitative determination of mouse 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.**

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INTRODUCTION

Interleukin 17, also known as cytotoxic T lymphocyte-associated antigen-8, is a 34 kDa disulfide-linked homodimeric glycoprotein that is produced primarily by activated mouse $\alpha\beta$ TCR⁺CD4⁻CD8⁻ thymocytes or human CD4⁺ memory T cells (1 - 4).

Mouse IL-17 cDNA encodes a 158 amino acid (aa) residue precursor protein with a 21 aa residue signal peptide that is cleaved to generate the 137 aa residue mature protein which contains six cysteine residues and a potential N-linked glycosylation site (1 - 4). The single copy gene for IL-17 has been mapped to mouse chromosome 1A. Mature mouse IL-17 shares 63% and 88% amino acid sequence identity with mature human and rat IL-17, respectively. IL-17 also exhibits a high degree of sequence homology to the putative protein encoded by the open reading frame (ORF) 13 gene of the T lymphotropic Herpes virus Saimiri (1).

IL-17 has been shown to have multiple biological effects *in vitro*. It can induce the release of a number of proinflammatory and/or hematopoietic cytokines (including TNF- α , IL-1 β , IL-6, IL-8, LIF, G-CSF and MCP-1) by adherent cells such as fibroblasts, keratinocytes, macrophages, epithelial and endothelial cells (1 - 7). IL-17 can also upregulate nitric oxide production by human articular chondrocytes (8, 9); induce ICAM-1 surface expression in fibroblasts; activate NF- κ B in fibroblasts and chondrocytes and stimulate proliferation of mouse splenic T cells (7). *In vivo*, IL-17 has been shown to stimulate hemopoiesis and secondary granulopoiesis in mice (10). Based on its *in vitro* and *in vivo* effects, it is likely that IL-17 is a significant regulator of T cell-dependent inflammatory reactions. IL-17 may also be an important T cell-derived mediator that links the immune system to hematopoiesis (7, 10).

An IL-17 receptor (IL-17 R), which binds IL-17 with low affinity, has been identified in mice and humans (11, 12). Mouse IL-17 R cDNA encodes an 864 aa residue type I transmembrane protein with a 291 residue extracellular domain and a 521 residue cytoplasmic tail. The extracellular and the cytoplasmic domains of IL-17 R share no homology with previously identified cytokine receptor families. In contrast to IL-17, whose expression is highly restricted, the expression of mouse IL-17 R has been detected in virtually all cells and tissues tested, including NK cells, macrophages, mast cells, pre-B cells, fibroblasts, fetal hepatocytes and intestinal epithelial cells (11, 12).

The Quantikine Mouse IL-17 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-17 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-17 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant mouse IL-17 accurately. Results obtained using natural mouse IL-17 showed linear curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that the Quantikine Immunoassay kit can be used to determine relative mass values for natural mouse IL-17.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-17 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse IL-17 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-17 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse IL-17 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in 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 receptors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

PRECAUTION

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

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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. Gently tap the plate frame. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED

Description	Part #	Cat. # M1700	Cat. # SM1700
Mouse IL-17 Microplates - 96 well microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-17.	890669	2 plates	6 plates
Mouse IL-17 Conjugate - 23 mL/vial of a polyclonal antibody against mouse IL-17 conjugated to horseradish peroxidase with preservatives.	890671	1 vial	3 vials
Mouse IL-17 Standard - 3.5 ng/vial of recombinant mouse IL-17 in a buffered protein base with preservatives; lyophilized.	890670	1 vial	3 vials
Mouse IL-17 Control - Recombinant mouse IL-17 in a buffered protein base with preservatives; lyophilized. The concentration range of mouse IL-17 after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	890672	1 vial	3 vials
Assay Diluent RD1-38 - 12.5 mL/vial of a buffered protein solution with preservatives.	895301	1 vial	3 vials
Calibrator Diluent RD5T - 21 mL/vial of a buffered protein solution with preservatives.	895175	1 vial	3 vials
Wash Buffer Concentrate - 50 mL/vial of a 25-fold concentrated solution of a buffered surfactant with preservatives.	895024	1 vial	3 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	3 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	3 vials
Stop Solution - 23 mL/vial of a diluted hydrochloric acid solution.	895174	1 vial	3 vials
Plate Covers - Adhesive strips.	640197	8 strips	24 strips

M1700 contains sufficient materials to run ELISAs on two 96 well plates.

SM1700 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PM1700). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use beyond kit expiration date.	
Opened/ Reconstituted Reagents	Mouse IL-17 Conjugate	May be stored for up to 1 month at 2 - 8° C.*
	Diluted Wash Buffer	
	Stop Solution	
	Calibrator Diluent RD5T	
	Assay Diluent RD1-38	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
	Mouse IL-17 Standard (700 pg/mL)	
	Mouse IL-17 Control	
	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.
- 1000 mL graduated cylinders.
- **Polypropylene** test tubes for dilution.

SAMPLE COLLECTION AND STORAGE

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

Serum - Allow blood samples to clot for 2 hours at room temperature or overnight at 2 - 8° C before centrifuging for 20 minutes at 2000 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 20 minutes at 2000 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 evaluated for use in this assay. Grossly hemolyzed or lipemic samples may not be suitable for measurement of mouse IL-17 with this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

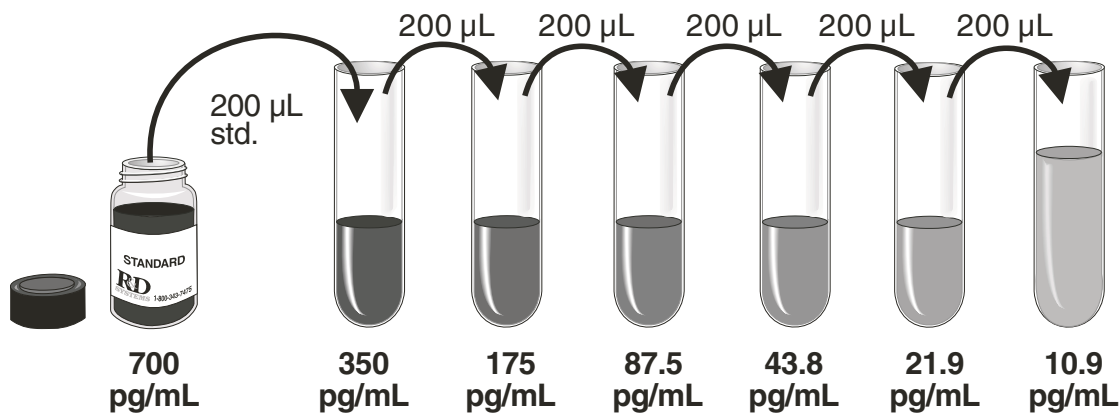
Mouse IL-17 Kit Control - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 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 μ L of the resultant mixture is required per well.

Mouse IL-17 Standard - Reconstitute the Mouse IL-17 Standard with 5.0 mL of Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 700 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions. Use this stock solution to produce a 2-fold dilution series (below).

Use polypropylene tubes. Pipette 200 μ L of Calibrator Diluent RD5T into each tube. The undiluted mouse IL-17 Standard serves as the high standard (700 pg/mL). Calibrator Diluent RD5T serves as the zero standard (0 pg/mL). Mix each tube thoroughly before the next transfer.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and control be assayed in duplicate.

1. Prepare all reagents, 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 RD1-38 to each well.
4. Add 50 μL of Standard, Control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. Plate layouts are provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
6. Add 100 μL of mouse IL-17 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 100 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

PROCEDURE SUMMARY AND CHECKLIST

1. Bring all reagents to room temperature.
 Prepare reagents, standards and samples as instructed.
 Return unused components to storage temperature as indicated in the instructions.

2. Add 50 μL of Assay Diluent to each well.

3. Add 50 μL of Standard, Control, or sample to each well.
 Tap plate gently for one minute.
 Cover the plate and incubate for 2 hours at room temperature.

4. Aspirate and wash each well five times.

5. Add 100 μL of Conjugate to each well.
 Cover the plate and incubate 2 hours at room temperature.

6. Aspirate and wash each well five times.

7. Add 100 μL of Substrate Solution to each well.
Incubate 30 minutes at room temperature.
Protect from light.

8. Add 100 μL of Stop Solution to each well.

9. Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

CALCULATION OF RESULTS

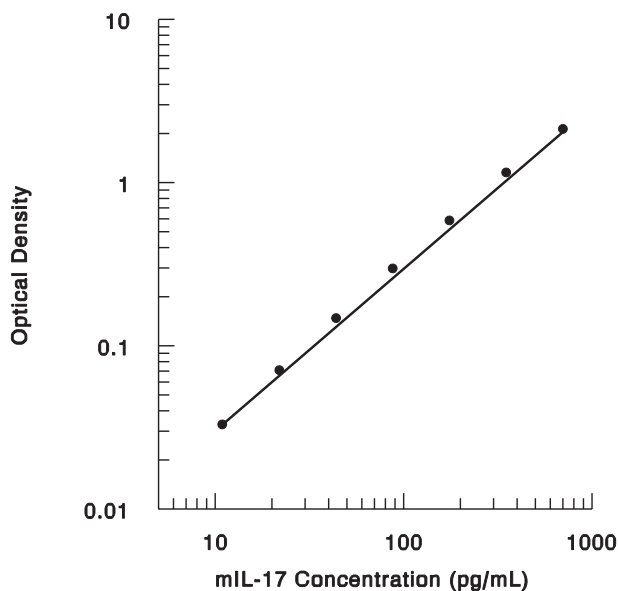
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 the graph. The data may be linearized by plotting the log of the mouse 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	0.032 0.032 0.064	0.032	—
10.9	0.066 0.103	0.065	0.033
21.9	0.103 0.177	0.103	0.071
43.8	0.183 0.321	0.180	0.148
87.5	0.339 0.604	0.330	0.298
175	0.634 1.158	0.619	0.587
350	1.213 2.123	1.186	1.154
700	2.204	2.164	2.132

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 assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	41.9	215	407	43.1	220	411
Standard deviation	2.1	6.3	8.1	2.2	11.2	18.5
CV (%)	5.0	2.9	2.0	5.1	5.1	4.5

RECOVERY

The recovery of mouse IL-17 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	101	92 - 118%
Mouse serum (n=6)	102	91 - 108%
Mouse heparin plasma (n=3)	101	91 - 123%
Mouse EDTA plasma (n=3)	107	93 - 120%

LINEARITY

To assess the linearity of the assay, five or more samples containing or spiked with various concentrations of mouse IL-17 in each matrix were diluted with Calibrator Diluent RD5T and then assayed. Results from typical sample dilutions are shown.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	Observed x 100 Expected
Cell Culture Supernates	neat	571		
	1/2	314	286	110%
	1/4	154	143	108%
	1/8	76	72	106%
	1/16	36	36	100%
Mouse Serum	spiked	618		
	1/2	314	309	102%
	1/4	153	154	99%
	1/8	75	77	97%
	1/16	36	38	95%
Mouse Heparin Plasma	spiked	448		
	1/2	244	224	109%
	1/4	126	112	113%
	1/8	64	56	114%
	1/16	33	28	118%
Mouse EDTA Plasma	spiked	472		
	1/2	259	236	110%
	1/4	133	118	113%
	1/8	70	59	119%
	1/16	34	30	113%

SENSITIVITY

The minimum detectable dose of mouse IL-17 was found to be typically less than 5 pg/mL.

The minimum detectable dose 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 mouse IL-17 produced at R&D Systems. This disulfide-linked homodimeric protein consists of two 137 amino acid residue monomers, each with a predicted molecular weight of 15.5 kDa. Based on total amino acid analysis, the absorbance of a 1 mg/mL solution of the *E. coli*-expressed recombinant mouse IL-17 at 280 nm was determined to be 1.00 A.U.

SAMPLE VALUES

Serum/Plasma - Forty mouse serum samples and six mouse plasma samples were evaluated for detectable levels of mouse IL-17 in this assay. All samples read below the lowest standard, 10.9 pg/mL.

Cell Culture Supernates - Mouse lung conditioned media (1 lung, 1 - 2 mm pieces in 10 mL DMEM containing 10% FCS and 5 µg/mL lipopolysaccharide) was collected after 6 days. The culture supernate was assayed for mouse IL-17 and measured 1800 pg/mL.

SPECIFICITY

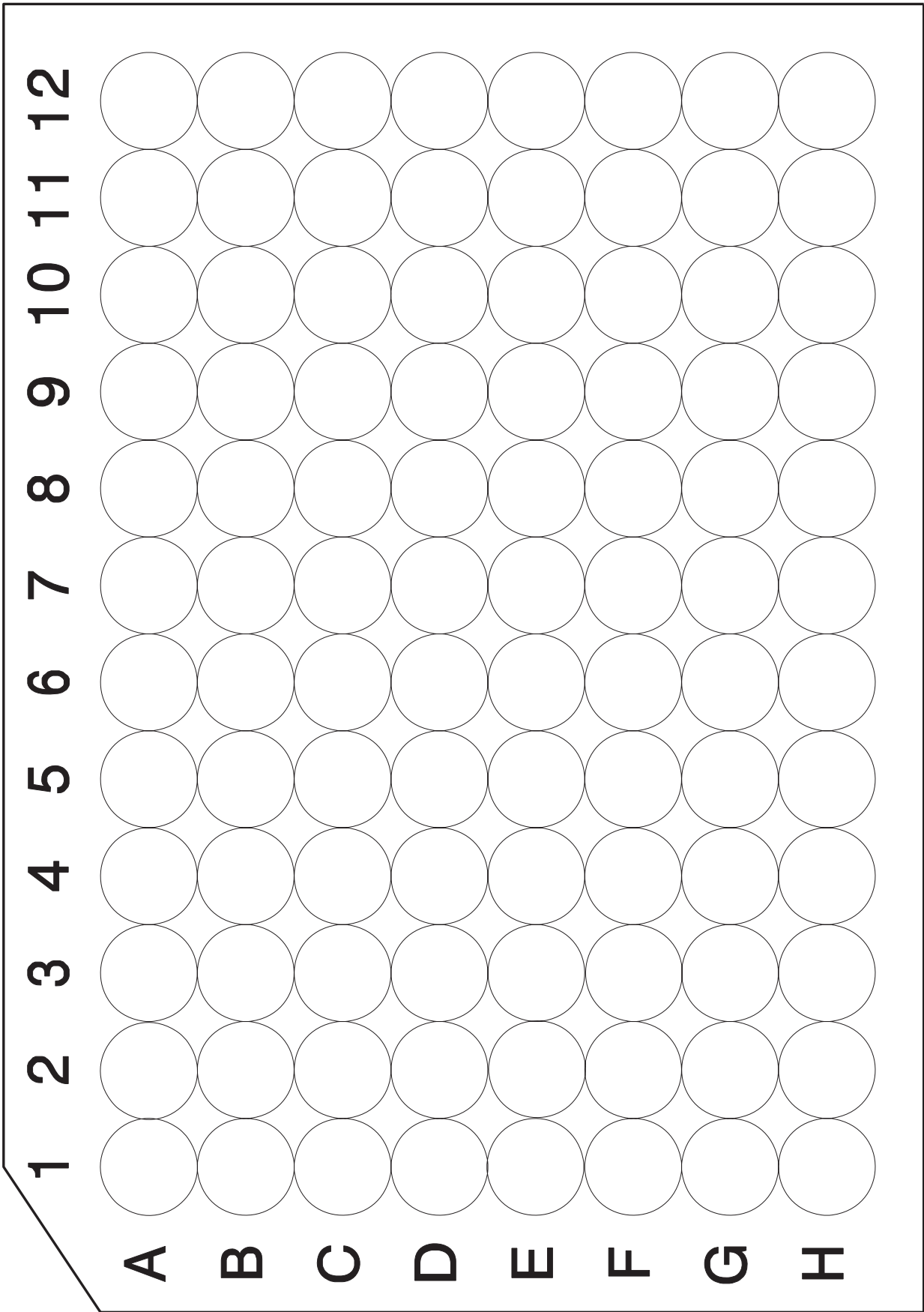
This assay recognizes both recombinant and natural mouse IL-17. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5T and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse IL-17 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10	IL-10	LIF
Eotaxin	IL-10 sR	M-CSF
G-CSF	IL-12	MIP-1 α
GM-CSF	IL-13	MIP-1 β
IFN- γ	IL-17B (aa 1 - 180)	MIP-2
IL-1 α	IL-17B (aa 21 - 180)	OSM
IL-1 β	IL-17B R	SCF
IL-1ra	IL-17D	TNF- α
IL-2	IL-17E	Tpo
IL-3	IL-17F	VEGF
IL-4	IL-17 R	
IL-5	IL-18	
IL-6	JE/MCP-1	Recombinant human:
IL-7	KC	IL-17
IL-9	Leptin	

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