Quantikine™ HS ELISA

Mouse IL-17 Immunoassay

Catalog Number MHS170

For the quantitative determination of mouse Interleukin 17 (IL-17) concentrations in 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

Mouse Interleukin 17 (IL-17; also known as IL-17A and CTLA-8) is a 21 kDa, variably glycosylated polypeptide that belongs to the IL-17 family of cytokines containing a cysteine-knot fold (1-3). Its sequence was originally isolated from an activated hybridoma created from the fusion of a mouse cytotoxic and rat T cell lymphoma cell line (2-5). It is synthesized as a 158 amino acid (aa) precursor that contains a 25 aa signal sequence and a 15 kDa, 133 aa mature segment (5). In both mouse and human, there is one conserved N-linked glycosylation site that likely contributes 5 kDa to its native molecular weight. IL-17A forms both a 35-38 kDa homodimer, and a 45-48 kDa heterodimer with IL-17F (6, 7). Mature mouse IL-17A is 61% and 89% aa identical to human and rat IL-17A, respectively (4, 5, 8). While rodent and human mature sequences show modest aa sequence identity, human IL-17 is active on both mouse and rat cells (5, 9). 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 (3, 5, 6, 10-12).

A high affinity receptor for mouse IL-17 has been reported, and appears to be a heteromultimer of IL-17RA and IL-17RC, likely in a 2:1 ratio (1). 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, 13). 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 (14, 15). 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, 16).

IL-17 is best known for its participation in the recruitment and survival of neutrophils (3, 10, 17, 18). Its induction was initially described to be the result of antigen stimulation of dentritic cells, resulting in IL-23 secretion. In a TCR-independent event, IL-23 induces T cell production of IL-17 (3). Once secreted, IL-17 in the bone marrow would seem to induce stromal/fibroblast expression of both G-CSF and SCF (membrane form), an effect that increases neutrophil differentiation and activation. IL-17 may complement this by directly blocking neutrophil apoptosis, promoting greater circulating neutrophil numbers (17). In the tissues, IL-17 seems 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 (19). TNF-α and IL-1β then act on local ECs to induce G-CSF secretion, an effect that is potentiated by IL-17 (20). IL-17 further contributes to neutrophil influx by inducing EC CXC chemokine release and NO production, which may increase vascular permeability (3, 9). IL-17 effects are not limited to neutrophils. In synovial joints, IL-17 upregulates RANKL expression on osteoblasts. This provides a stimulus for osteoclast formation and subsequent bone resorption (18).

The Quantikine™ HS Mouse IL-17 Immunoassay is a 4.0 hour solid phase ELISA designed to measure mouse IL-17 levels in serum and plasma. It contains *E. coli*-expressed recombinant mouse IL-17 and antibodies raised against the recombinant protein. Results obtained using natural IL-17 showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit standards. These results indicate that the Quantikine HS 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 and samples are pipetted into the wells and any IL-17 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for mouse IL-17 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-17 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.
- 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

- To ensure accurate results, bring liquids to room temperature and mix to homogeneity prior to pipetting or aliquoting.
- When mixing protein solutions, always avoid foaming.
- 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
Mouse IL-17 HS Microplate	899259	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-17.	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.*
Mouse IL-17 HS Standard	899261	2 vials of recombinant mouse IL-17 in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard after use.
Mouse IL-17 HS Conjugate	899260	21 mL of a polyclonal antibody specific for mouse IL-17 conjugated to biotin with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-38	895301	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a buffered protein base 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.*	
Stop Solution	895032	6 mL of 2N sulfuric acid.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Streptavidin Polymer-HRP Diluent	898387	21 mL of a solution with preservatives.	
Streptavidin Polymer-HRP (100X)	898350	0.3 mL of Streptavidin Polymer-HRP in a buffer with preservative.	
Plate Sealers	N/A	8 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
- 1000 mL graduated cylinder
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Mouse IL-17 Controls HS (optional; R&D Systems®, Catalog # QC292)

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.

Serum - Allow blood samples to clot for 2 hours at room temperature 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: *Grossly hemolyzed samples are not suitable for use in this assay.*
Citrate plasma has not been validated for use in this assay.

SAMPLE PREPARATION

Multiple dilutions are recommended for unknown samples.

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 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

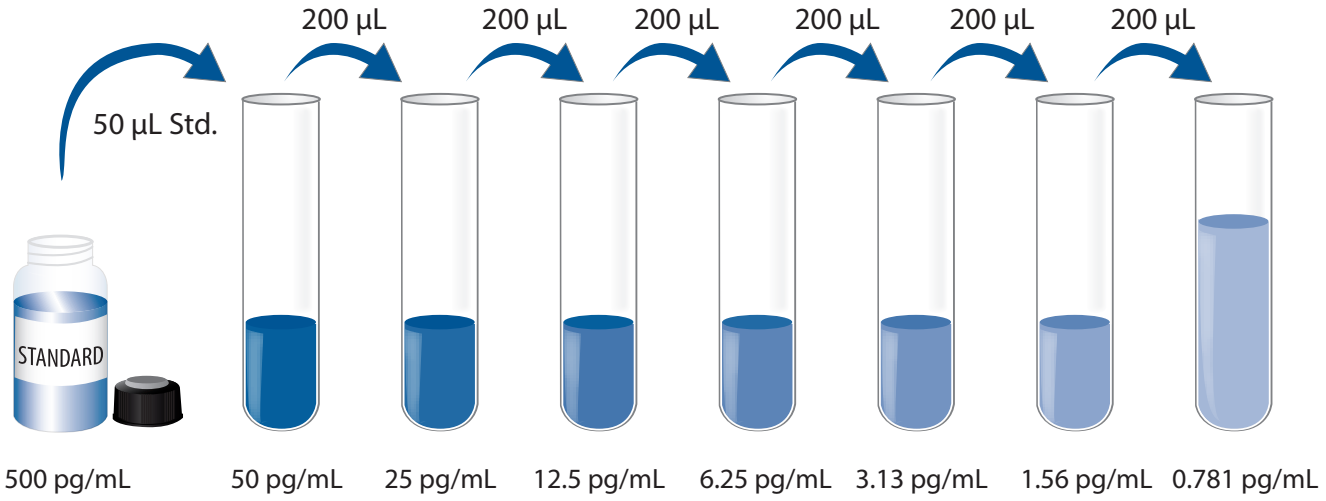
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

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).

Streptavidin Polymer-HRP (1X) - Add 0.215 mL of Streptavidin Polymer-HRP (100X) directly to the Streptavidin Polymer-HRP Diluent. Mix well.

Mouse IL-17 HS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-17 HS Standard with deionized or distilled water. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 50 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 50 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 standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents and working standards 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. 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.
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 Mouse IL-17 HS Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the wash as in step 5.
8. Add 200 µL of Streptavidin Polymer-HRP (1X) to each well. Cover with a new adhesive strip. Incubate for **30 minutes** at room temperature on the shaker.
9. Repeat the wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for **30 minutes** at room temperature **on the benchtop. Protect from light.**
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.

CALCULATION OF RESULTS

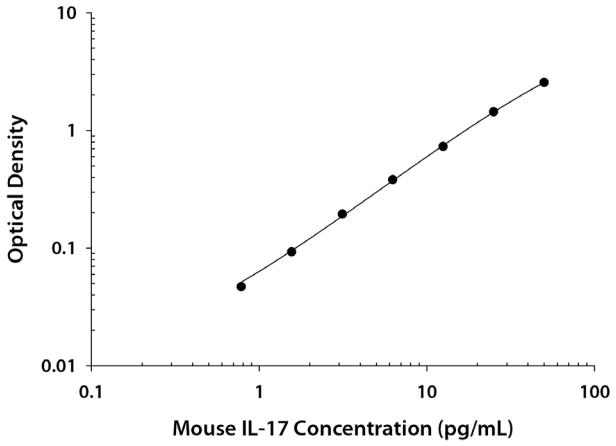
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 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 measured concentrations 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	0.022 0.023	0.023	—
0.781	0.069 0.070	0.070	0.047
1.56	0.114 0.118	0.116	0.093
3.13	0.212 0.223	0.218	0.195
6.25	0.388 0.421	0.405	0.382
12.5	0.752 0.757	0.755	0.732
25	1.425 1.501	1.463	1.440
50	2.575 2.584	2.580	2.557

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	6.82	16.9	31.9	6.92	16.6	31.4
Standard deviation	0.239	0.429	0.836	0.349	0.814	1.64
CV (%)	3.5	2.5	2.6	5.1	4.9	5.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Serum (n=4)	89	75-98%
EDTA plasma (n=4)	90	78-96%
Heparin plasma (n=4)	89	80-97%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse IL-17 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	100	100	102
	Range (%)	94-103	99-102	94-112
1:4	Average % of Expected	101	105	105
	Range (%)	95-109	103-108	99-115
1:8	Average % of Expected	106	111	111
	Range (%)	97-118	107-113	103-122
1:16	Average % of Expected	116	116	110
	Range (%)	103-125	113-121	105-116

SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of mouse IL-17 ranged from 0.043-0.214 pg/mL. The mean MDD was 0.107 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 *E. coli*-expressed recombinant mouse IL-17 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IL-17 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	3.54	0.795-13.0	3.95
EDTA plasma (n=5)	1.97	0.959-3.60	1.05
Heparin plasma (n=5)	2.24	1.25-3.72	0.920

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-17.

The factors listed below were prepared at 500 pg/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 500 pg/mL in a mid-range recombinant mouse IL-17 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-17B
IL-17B R
IL-17D
IL-17E
IL-17F
IL-17 R
IL-17RC

Recombinant human:

human IL-17A

Some cross-reactivity was observed with recombinant mouse IL-17 A/F heterodimer.

Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-Reactivity
100	7.96	7.96

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