Quantikine™ ELISA

Mouse IL-17A/F Heterodimer Immunoassay

Catalog Number M17AF0

For the quantitative determination of mouse IL-17A/F Heterodimer 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

Mouse IL-17A/F is an approximately 40 kDa, secreted, disulfide-linked heterodimeric glycoprotein comprised of two members of the IL-17 family of cytokines, IL-17A and IL-17F (1). Members of this family demonstrate a structural motif termed a cysteine knot that also characterizes a large superfamily of growth factors. Although most cysteine knot superfamily members use three intrachain disulfide bonds to create a knot, IL-17 family molecules generate the same structural form with only two disulfide links (2-4). Mouse IL-17A is synthesized as a 158 amino acid (aa) precursor that contains a 25 aa signal sequence and a 133 aa mature chain. The mature chain contains one potential site for N-linked glycosylation. Mouse IL-17F is synthesized as a 161 aa precursor with a 29 aa signal sequence and a 132 aa mature chain. Like IL-17A, the mature chain of IL-17F contains one potential site for N-linked glycosylation. IL-17A and IL-17F share 50% aa sequence homology and are syntenic, both located at the A4 region of mouse chromosome 1 (5). Mouse IL-17A and IL-17F share 61% and 56% aa sequence identity with human IL-17A and IL-17F, respectively.

IL-17A/F and the IL-17A and IL-17F homodimers are produced by an activated subset of $CD4^+$ T-cells, termed Th17 (1, 5-7). IL-23 drives Th17 lymphocytes to produce these cytokines (6, 8-9). Recent studies have identified that the widely expressed receptors IL-17RA and IL-17RC form a heterodimer for the binding of IL-17A and IL-17F, and it is postulated that IL-17A/F also binds IL-17RA (6, 10-11).

IL-17A expression is associated with many inflammatory diseases in humans, such as rheumatoid arthritis, multiple sclerosis, asthma, systemic lupus erythematosus, and allograft rejection (11). *In vitro*, IL-17A regulates inflammatory responses by inducing the expression of IL-6, GROα, GM-CSF, several chemokines (CCL2, CCL7, CCL20, and CXCL1), and matrix metalloproteinases (MMP-3 and MMP-13) (4, 11-12). Expression of IL-17F is also linked with human inflammatory diseases, including asthma (11, 13). Treatment of human airway epithelial cells, fibroblasts, and vein endothelial cells with IL-17F induces the expression of IL-6, IL-8, GROα, ENA-78, TGF-β, CCL2, G-CSF, GM-CSF, and ICAM-1 (11). IL-17A/F, on its own or in synergy with TNF-α, regulates the expression of IL-6 and CXCL1 (the mouse homolog of human GROα), which is dependent on IL-17RA and TRAF6 (11). IL-17A/F induces chemokine production and airway neutrophilia with intermediate potency between IL-17A (most potent) and IL-17F (least potent) (5).

The Quantikine™ Mouse IL-17A/F Heterodimer Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-17A/F heterodimer in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse mature IL-17A/F heterodimer and antibodies raised against the recombinant homodimer factors. This immunoassay has been shown to accurately quantitate the recombinant mouse IL-17A/F heterodimer. Results obtained using natural mouse IL-17A/F heterodimer showed dose response 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 mouse IL-17A/F heterodimer.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-17A has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-17A/F heterodimer present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-17F 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 IL-17A/F heterodimer 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 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.
- For best results, pipette reagents and samples into the center of each well.
- 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.

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-17A/F Microplate	890669	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-17A.	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-17A/F Conjugate	893738	12 mL of a polyclonal antibody specific for mouse IL-17F conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse IL-17A/F Standard	893739	Recombinant mouse IL-17A/F heterodimer in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse IL-17A/F Control	893740	Recombinant mouse IL-17A/F heterodimer in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	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	895174	23 mL of diluted hydrochloric 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
- 500 mL graduated cylinder
- **Polypropylene** test tubes for dilution of standards

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. 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 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 validated for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

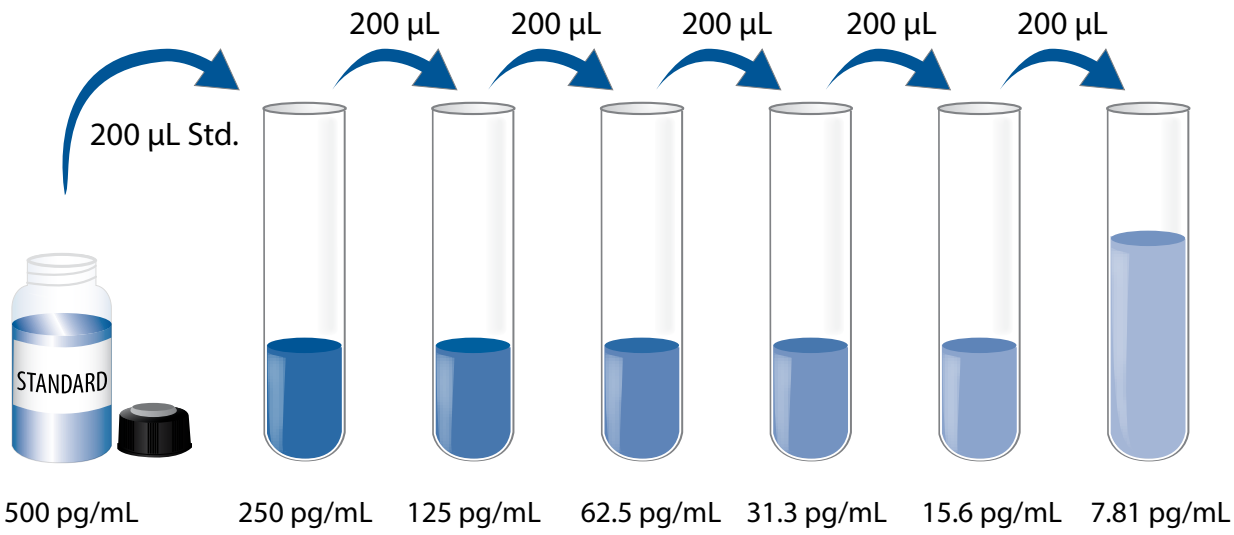
Mouse IL-17A/F Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. 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.

Mouse IL-17A/F Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-17A/F Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-3 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-17A/F Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 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, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, 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, reseal.
3. Add 50 µL of Assay Diluent RD1W 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.
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 decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse IL-17A/F 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.

CALCULATION OF RESULTS

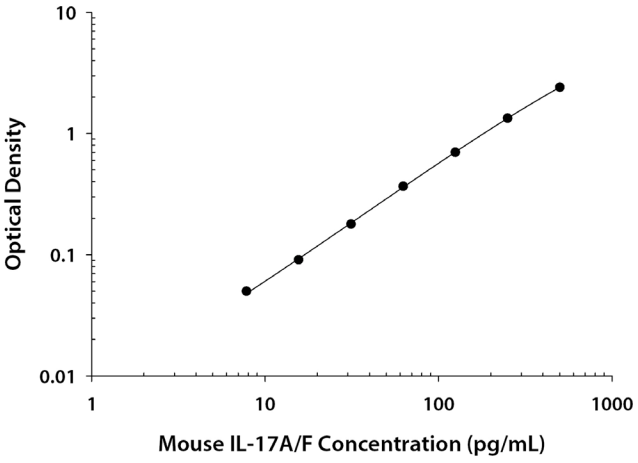
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-17A/F 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	0.051 0.054	0.053	—
7.81	0.102 0.103	0.103	0.050
15.6	0.141 0.146	0.144	0.091
31.3	0.229 0.234	0.232	0.179
62.5	0.418 0.421	0.420	0.367
125	0.746 0.760	0.753	0.700
250	1.349 1.431	1.390	1.337
500	2.421 2.499	2.460	2.407

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. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	22.8	119	180	25.1	112	188
Standard deviation	1.4	5.0	11.1	2.0	9.8	11.6
CV (%)	6.1	4.2	6.2	8.0	8.8	6.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	105	93-120%
Serum (n=4)	91	84-98%
EDTA plasma (n=4)	99	90-104%
Heparin plasma (n=4)	91	86-98%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse IL-17A/F 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)
1:2	Average % of Expected	99	109	99	103
	Range (%)	97-102	105-111	91-104	99-108
1:4	Average % of Expected	103	111	99	102
	Range (%)	100-105	103-115	90-105	97-109
1:8	Average % of Expected	104	111	104	101
	Range (%)	100-110	108-115	94-115	94-107
1:16	Average % of Expected	106	115	99	101
	Range (%)	100-112	113-117	93-102	87-118

SENSITIVITY

Fifty-nine assays were evaluated and the minimum detectable dose (MDD) of mouse IL-17A/F heterodimer ranged from 0.3-2.8 pg/mL. The mean MDD was 1.4 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-17A/F heterodimer produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	69.4	30	ND-349
EDTA plasma (n=20)	36.4	50	ND-264
Heparin plasma (n=20)	51.2	40	ND-322

ND=Non-detectable

Cell Culture Supernates - Two mouse spleens were homogenized and seeded into 100 mL of RPMI containing 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 18, 48, and 72 hours. The cells were cultured unstimulated or stimulated with 10 ng/mL Concanavalin A (ConA) or 1 µg/mL lipopolysaccharide (LPS). Aliquots of the cell culture supernates were removed and assayed for levels of mouse IL-17A/F heterodimer.

Condition	18 hours (pg/mL)	48 hours (pg/mL)	72 hours (pg/mL)
Unstimulated	——	ND	33.7
Stimulated with 10 ng/mL ConA	377	3126	——
Stimulated with 1 µg/mL LPS	——	127	109

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-17A/F heterodimer.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors in a mid-range mouse IL-17A/F heterodimer control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

G-CSF
IL-2
IL-6
IL-15
IL-17B
IL-17B R
IL-17C
IL-17D
IL-17E
IL-17F
IL-17 R
IL-17 RC
IL-17 RD
IL-23
TGF-β RII

Recombinant rat:

IL-17F

Recombinant human:

IL-17A
IL-17A/F Heterodimer
IL-17C
IL-17D
IL-17F

Recombinant mouse IL-17A does not interfere but does cross-react approximately 0.07% in this assay.

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