Murine IL-17F ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 660.100.096

For research use only

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Murine IL-17F ELISA KIT

1. Intended use

The mouse IL-17F ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of mouse IL-17F. **The mouse IL-17F ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

2. Introduction

2.1. Summary

Interleukins 17F (IL-17F) and 17A (IL-17A) are closely related members of the IL-17 cytokine family, sharing about 50% amino acid identity. Studies in the mouse have identified Th17 cells as a distinct CD4+ T cell lineage that is defined by the production of IL-17F and IL-17A. IL-6 and transforming growth factor-β are required for the differentiation of naïve CD4+ T cells to Th17 cells, which are maintained in the presence of IL-23 and IL-1β. Conversely, IL-4 and interferon-γ can inhibit the development of Th17 cells. Th17 cells have been implicated in the pathology of mouse autoimmune disease models.

Expression of IL-17F and IL-17A has been detected in activated human peripheral blood lymphocytes. It has been shown that the cytokines are expressed in activated human CD4+ T cells. Expression of IL-17F and IL-17A has also been observed in tissue samples from various autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis, inflammatory bowel disease, and asthma.

The crystal structure of IL-17F has been solved and shows that the protein forms a disulfide-linked dimeric glycoprotein. IL-17A is also a disulfide-linked homodimeric glycoprotein. The IL-17F homodimer includes a classical cysteine knot motif, which is found in the TGF-β, bone morphogenetic protein, and nerve growth factor superfamilies. One difference in the cysteine knot motif of IL-17F compared with the other known cysteine knot protein families is that it only utilizes four cysteines instead of the classical six cysteines to form the knot. IL-17F and IL-17A have been shown to form biologically active IL-17F/IL-17A heterodimers, in addition to the IL-17F and IL-17A homodimers.

2.2. Principle of the method

An anti-mouse IL-17F coating antibody is adsorbed onto microwells.
Mouse IL-17F present in the sample or standard binds to antibodies adsorbed to the microwells.
Following incubation unbound biological components are removed during a wash step and a biotin-conjugated anti-mouse IL-17F antibody is added and binds to mouse IL-17F captured by the first antibody.
Following incubation unbound biotin-conjugated anti-mouse IL-17F antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-mouse IL-17F antibody.
Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.
A coloured product is formed in proportion to the amount of mouse IL-17F present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 mouse IL-17F standard dilutions and mouse IL-17F sample concentration determined.

3. Reagents provided and reconstitution

REAGENTS (store at 2-8°C)	Quantity 1x96 well kit 660.100.096	RECONSTITUTION
96-wells precoated microtiter plate	1	Ready-to-use
Plate covers	2	
Biotin-Conjugate anti-mouse IL-17F polyclonal antibody	1 vial	Dilute 100 times in Assay Buffer (120µl)
Streptavidin-HRP	1 vial	Dilute 100 times in Assay Buffer (150µl)
mIL-17F Standard: 4 ng/ml	2 vials	Reconstitute as indicated on the vial
Conjugate Diluent	1 vial	(15 ml) Ready-to-use
Sample Diluent	1 vial	(12 ml) Ready-to-use
Assay Buffer	1 vial	(5 ml) 20X concentrate. Dilute in distilled water.
Wash Buffer	1 vial	(50 ml) 20X concentrate. Dilute in distilled water
Calibrator Diluent	1 vial	(5 ml) Ready-to-use
Substrate Solution	1 vial	(15 ml) Ready-to-use
Stop Solution (1M Phosphoric acid)	1 vial	(15 ml) Ready-to-use

4. Materials required but not provided

- Microtitre plate reader fitted with appropriate filters (450nm required with optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Storage Instructions

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. Specimen collection, processing & storage

Cell culture supernatant, serum and plasma (EDTA) were tested with this assay. Other body fluids might be suitable for use in the assay. Remove serum from the clot as soon as possible after clotting.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive mouse IL-17F. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Do not thaw samples in a 37°C water bath. Do not vortex or sharply agitate samples.

7. Safety & precautions for use

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual : "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance’s within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution within 15 min of the washing of the microtiter plate.

8. Assay Preparation

Bring all reagents to room temperature before use.
If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

8.1. Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

Example plate layout (example shown for a 6 points standard curve)

	Standards (pg/ml)		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	4000	4000										
B	2000	2000										
C	1000	1000										
D	500	500										
E	250	250										
F	125	125										
G	Zero	Zero										
H												

All remaining empty wells can be used to test samples in duplicate

8.2. Preparation of Wash Buffer

Pour entire contents (50 ml) of the Wash Buffer Concentrate (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water.
Mix gently to avoid foaming.
Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

8.3. Preparation of Assay Buffer

Pour the entire contents (5 ml) of the **Assay Buffer Concentrate** (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.
Store at 2° to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

8.4. Preparation of Standard

Reconstitute **mouse IL-17F standard** by addition of Calibrator diluent.

Reconstitution volume is stated on the label of the standard vial. Allow the reconstituted standard to sit for 10-30 minutes. Swirl or mix gently to insure complete and homogeneous solubilisation (concentration of reconstituted standard = 4 ng/ml).

Label 6 tubes, one for each standard point. S2, S3, S4, S5, S6, S7
The reconstituted standard serves as S1.
Then prepare 1:2 serial dilutions for the standard curve as follows:
Pipette 150 µl of Calibrator Diluent into each tube S2-S7.
Pipette 150 µl of reconstituted standard (concentration = 4 ng/ml) into the first tube, labelled S2, and mix (concentration of standard 2 = 2 ng/ml).
Pipette 150 µl of this dilution into the second tube, labelled S3, and mix thoroughly before the next transfer.
Repeat serial dilutions 4 more times thus creating the points of the standard curve ranging from 4000 to 62.5 pg/ml.

Calibrator Diluent serves as Blank

8.5. Preparation of Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** solution with conjugate diluent in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Conjugate Diluent (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

8.6. Preparation of Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

9. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.

Prepare all reagents as shown in section 8.
Note: Final preparation of Biotin conjugate (section 8.5) and Streptavidin-HRP (section 8.6) should occur immediately before use.

Assay Step		Details
1.	Wash	a) Dispense 0.4 ml of **1x washing solution** into each well b) Aspirate the contents of each well c) Repeat steps a and b Do not allow wells to dry before use
2.	Addition	Add 50µl of **Sample diluent** to all wells
3.	Preparation	**Prepare Standard curve** as shown in section 8.4
4.	Addition	Add 50µl of each **Standard, Sample and Blank (Calibrator Diluent)** in duplicate to appropriate number of wells
5.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **2 hour(s)** if available on a microplate shaker set at 400 rpm
6.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.4 ml of **1x washing solution** into each well c) Aspirate the contents of each well d) Repeat step b and c another four times
7.	Addition	Add 100µl of diluted **biotin-conjugate** to all wells
8.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour(s)** if available on a microplate shaker set at 400 rpm
9.	Wash	Repeat wash step 6.
10.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells
11.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour** if available on a microplate shaker set at 400 rpm
12.	Wash	Repeat wash step 6.
13.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
14.	Incubation	Incubate in the dark for **30 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
15.	Addition	Add 100µl of **Stop Reagent** into all wells
Read the absorbance value of each well (immediately after step 15.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).		

*****_Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range_

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

10. Data Analysis

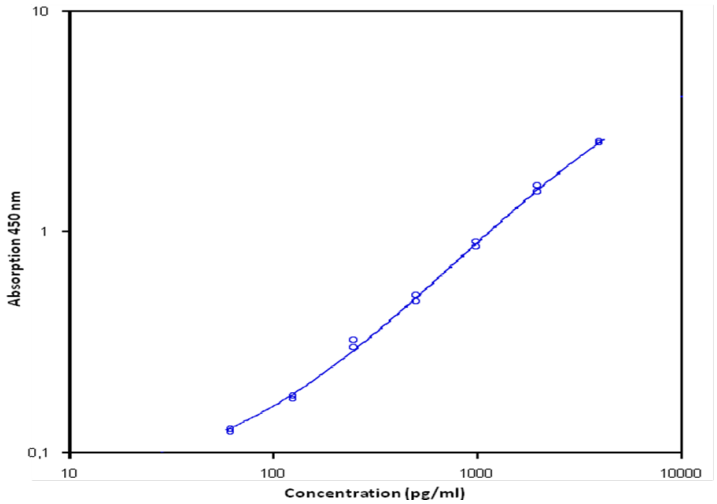
Calculate the average absorbance values for each set of duplicate standards and samples**.** Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding mIL-17F standard concentration on the horizontal axis.

The amount of mIL-17F in each sample is determined by extrapolating OD values against mIL-17F standard concentrations using the standard curve**.**

Example mIL-17F Standard Curve:

Standard	mIL-17F Conc (pg/ml)	OD (450nm) mean	CV (%)
1	4000.0	2.528	1.0
2	2000.0	1.554	3.3
3	1000.0	0.875	2.6
4	500.0	0.496	3.1
5	250.0	0.309	3.6
6	125.0	0.176	1.8
Blank	0	0.056	0.7



Note: curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed

11. Assay limitations

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

12. Performance Characteristics

12.1. Sensitivity

The limit of detection of mouse IL-17F defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 10.0 pg/ml (mean of 6 independent assays).

12.2. Specificity

The assay detects both natural and recombinant mouse IL-17F.
The cross reactivity and interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a mouse IL-17F positive sample.
No cross reactivity to the IL-17AA homodimer is observed and less than 2.5% cross reactivity to IL-17AF heterodimer when spiked in excess (100ng/ml).

12.3. Precision

Intra Assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of mouse IL-17F. 2 standard curves were run on each plate. **The calculated overall intra-assay coefficient of variation was 4.2%.**

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of mouse IL-17F. 2 standard curves were run on each plate. **The calculated overall inter-assay coefficient of variation was 4.9%.**

12.4. Dilution Parallelism

Serum, plasma and cell culture supernatant samples with different levels of mouse IL-17F were analysed at serial 2 fold dilutions with 4 replicates each.

Sample matrix	Recovery Range (%)	Mean (%)
Serum	102 - 133	112
Plasma (EDTA)	96 - 119	107
Cell culture supernatant	76 - 106	89

12.5. Spike Recovery

The spike recovery was evaluated by spiking 3 levels of mouse IL-17F into serum, plasma and cell culture supernatant. Recoveries were determined with 4 replicates each. The unspiked serum, plasma or cell culture supernatant was used as blank in these experiments.

Sample matrix	Spike high (%)	Spike medium (%)	Spike low (%)
Serum	105	100	107
Plasma (EDTA)	116	114	139
Cell culture supernatant	99	95	100

12.6. Stability

Freeze-Thaw Stability

Aliquots of serum samples (spiked) were stored at -20°C and thawed 3 times, and the mouse IL-17F levels determined. There was no significant loss of mouse IL-17F immunoreactivity detected by freezing and thawing.

Storage Stability

Aliquots of serum samples (spiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the mouse IL-17F level determined after 24 h. There was no significant loss of mouse IL-17F immunoreactivity detected during storage under above conditions.
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12.7. Expected Values

Panels of 40 serum as well as EDTA plasma samples from randomly selected apparently healthy mice were tested for mouse IL-17F.
There were no detectable mouse IL-17F levels found.
Elevated mouse IL-17F levels depend on the type of immunological disorder.

13. Assay Summary

Total procedure length : 4h30mn

Wash two times

↓

Add sample, diluted standard

↓

Incubate 2 hours at room temperature

↓

Wash five times

↓

Add 100µl of a Biotin-Conjugate

↓

Incubate 1hour at room temperature

↓

Wash five times

↓

Add 100µl of Streptavidin-HRP

↓

Incubate 1hour at room temperature

↓

Wash five times

↓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 30 mn.

↓

Add 100 µl Stop Reagent

↓

Read Absorbance at 450 nm

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