Mouse IL-17A ELISA Kit

For the quantitative determination of mouse interleukin-17A (mIL-17A) concentrations in cell culture supernatant and mouse serum

Catalogue Number: MEC1001

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca
Secure Online Store: www.anogen.net

S7.5 (01) mIL-17A

TABLE OF CONTENTS

INTENDED USE

This mouse Interleukin-17A ELISA Kit is designed for the *in vitro* quantitative determination of mouse interleukin-17A (IL-17A) concentrations in cell culture supernatant and mouse serum. This kit is intended for LABORATORY RESEARCH ONLY.

INTRODUCTION

Mouse Interleukin -17A (mIL-17A), also know as CTLA-8, is a secreted, homodimeric glycoprotein linked by disulfide link with a molecular mass of about 35 KD. The cytokine is the proto-type of a newly discovered pro-inflammatory cytokine family which consists of IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17A is mainly produced by a group of CD4 T helper cells termed Th17 cells. In addition to Th17 cells, CD8 T helper cells, natural killer cells and granulocytes, can also secret the cytokine after proper stimulation. IL17A exhibits structural homology and cross-species bioactivity between human and mouse cells.

IL-17A acts through its receptor IL-17R. It was found to stimulate the expression of pro-inflammatory cytokines including IL-6, MCP-1, G-CSF, GM-CSF, IL-1alpha and TNF-alpha. Most of those cytokines plays a role in the proliferation, maturation and chemotaxis of neutrophils. Elevated levels of IL-17A have been found to implicate with autoimmune diseases, airway inflammation, allograft rejection, inflammatory bowel disease, psoriasis, cancer, vascular inflammation and development of atherosis.

This mouse IL-17A ELISA is a 3.5-hour solid phase immunoassay readily applicable to measure mouse IL-17A levels in cell culture supernatant and mouse serum in the range of 0 to 1000 pg/mL. It showed no cross reactivity with other cytokines tested. This mouse IL-17A ELISA is expected to be effectively used for further investigations into the relationship between IL-17A and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This mouse IL-17A enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to mouse IL-17A. Standards or samples are then added to the appropriate microtiter plate wells. A biotin-conjugated antibody preparation specific for mouse IL-17A was added and incubated. Mouse IL-17A, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound mouse IL-17A and other components in the sample. Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. The wells are thoroughly washed to remove all unbound HRP-conjugated Avidin, and a TMB (3,3'5, 5' tetramethyl-

benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain mouse IL-17A, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of mouse IL-17A in the samples this kit includes two calibration diluents (Calibrator Diluent I for serum testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus mouse IL-17A concentration (pg/mL). The concentration of IL-17A in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- The kit should not be used beyond the expiration date on the kit label.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED
All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **Anti- MOUSE IL-17A MICROTITER PLATE** (Part MEC01-1) ***96 wells***
   Pre-coated with anti-mouse IL-17A monoclonal antibody.

2. **BIOTIN CONJUGATE** (Part MEC01-2) ***6 mL***
   Anti-mouse IL-17A antibody conjugated to Biotin.

3. **AVIDIN CONJUGATE** (Part MEC01-3) ***12 mL***
   Avidin conjugated to horseradish peroxidase.

4. **MOUSE IL-17A STANDARD** (Part MEC01-4) ***2 vials***
   Recombinant mouse IL-17A (1ng/vial) in a buffered protein base with preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part MEC01-5) ***25 mL***
   Newborn calf serum with buffer and preservative. *For serum/plasma testing*

6. **CALIBRATOR DILUENT II** (Part MEC01-6) ***25 mL***
   Cell culture medium with newborn calf serum and preservative. *For cell culture supernatant testing*

7. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part MEC01-7) ***10 mL***
   Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB

10. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4) Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. All biological waste should be disposed of in a manner that will inactivate infectious agents.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes before disposal.
9. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
10. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
11. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

- Serum and cell culture supernatant samples to be used immediately after collection, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

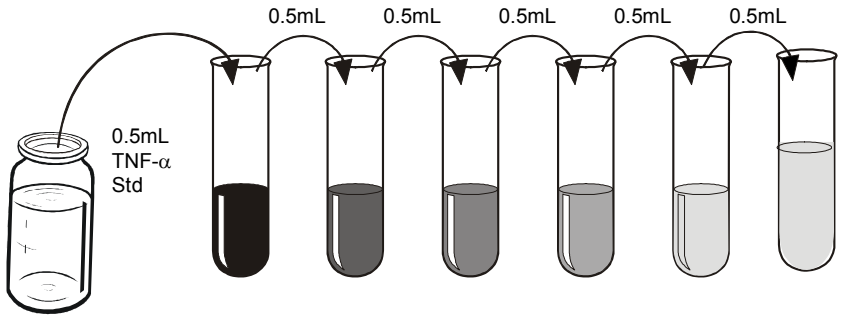
Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **Mouse IL-17A Standard:**

a) Two vials of Standard are provided in this kit to allow both serum and cell culture supernatant testing. Reconstitute mouse IL-17A Standard with 1.0 mL of Calibrator Diluent I or Calibrator Diluent II. This reconstitution produces a stock solution of 1000 pg/mL. Allow solution to sit for 5 minutes with gentle agitation prior to making dilutions. Use immediately after reconstituting. The mouse IL-17A standard stock solution can be

stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (15.6 to 1000pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted mouse IL-17A stock solution will serve as the high standard **(**1000 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



mIL-17A Standard	500 pg/ml	250 pg/ml	125pg/ml	62.5 pg/ml	31.2 pg/ml	15.6pg/ml	1000 pg/ml

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and mouse IL-17A Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1- **0 pg/mL** (S1)	**2C, 2D**	Standard 6- **250 pg/mL** (S6)
1C, 1D	Standard 2- **15.6 pg/mL** (S2)	**2E, 2F**	Standard 7- **500 pg/mL** (S7)
1E, 1F	Standard 3- **31.2 pg/mL** (S3)	**2G, 2H**	Standard 8- **1000 pg/mL** (S8)
1G, 1H	Standard 4- **62.5 pg/mL** (S4)	**3A, 3B**	Samples
2A, 2B	Standard 5- **125 pg/mL** (S5)		

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50μL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate 1 hour at room temperature.

3. Without discarding the standards and samples, add 50μL Anti-mouse IL-17A Biotin conjugate to each wells. Mix well. Cover and incubate for 1 hour at room temperature.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100μl of Avidin Conjugate to each well Mix well. Cover and incubate for 1 hour at room temperature.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.

8. Add 100μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

9. Add 100μL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of mouse IL-17A in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding mouse IL-17A concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 pg/mL)) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of mouse IL-17A in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-17A concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

3.

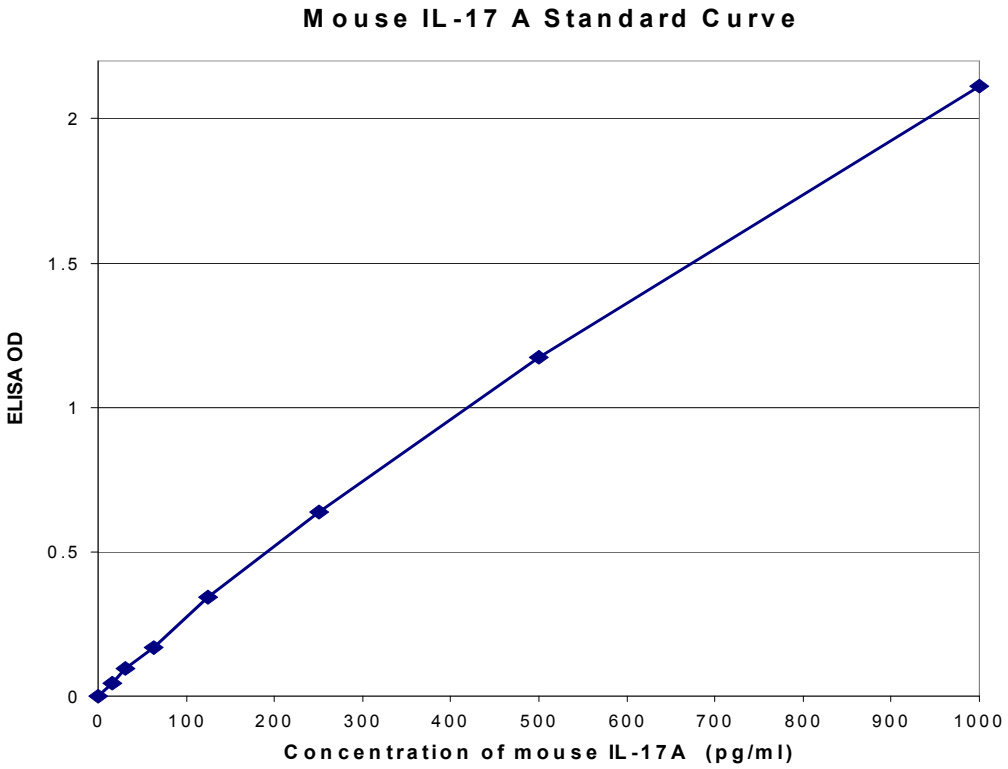
TYPICAL DATA

Results of a typical standard run of a mouse IL-17A ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain own standard curve with each run.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibrator Diluent I.

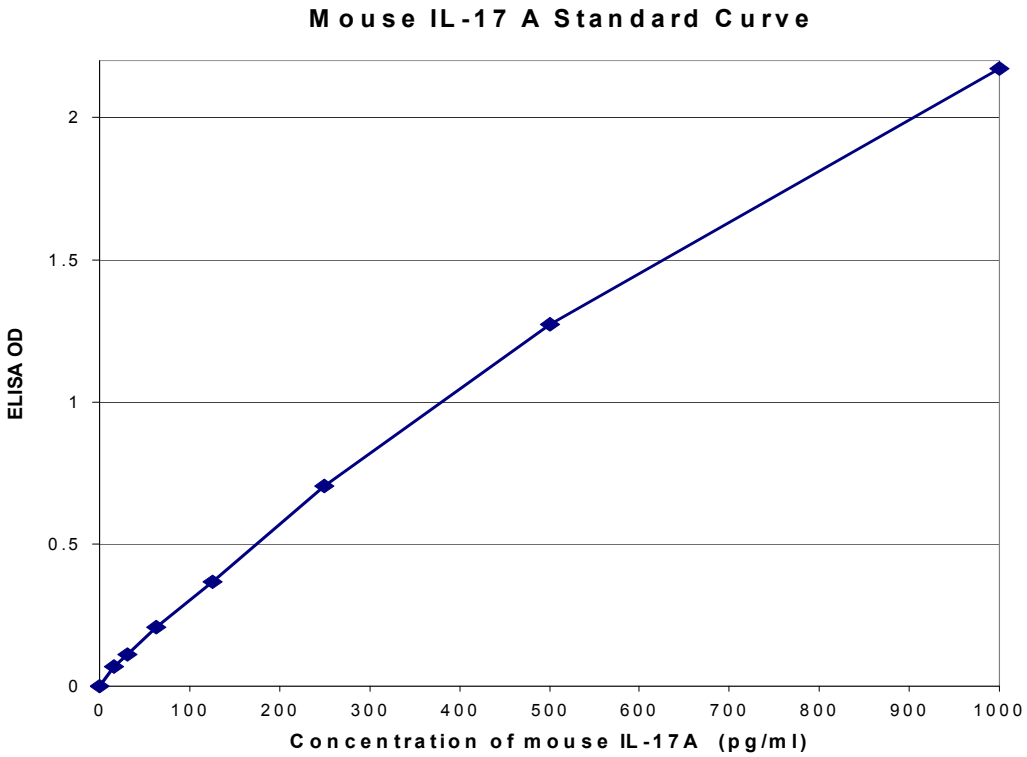
Standard (pg/mL)	Mean O.D. (450 nm)	CV	Zero Standard Subtracted (Std.) - (S1)
0	0.094	0.77	0.000
15.6	0.139	0.89	0.045
31.2	0.191	0.87	0.097
62.5	0.264	0.17	0.170
125	0.407	1.34	0.343
250	0.733	0.00	0.639
500	1.267	3.55	1.173
1000	2.207	1.35	2.113



EXAMPLE TWO

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	CV	Zero Standard Subtracted (Std.) - (S1)
0	0.055	2.570	0.000
15.6	0.125	0.000	0.070
31.2	0.165	1.280	0.110
62.5	0.263	1.610	0.208
125	0.422	1.340	0.367
250	0.759	1.860	0.704
500	1.329	1.600	1.274
1000	2.229	0.634	2.174



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To determine within-run precision in Calibrator II, three different samples of known concentration were assayed by replicates of eight in one assay.

Sample	**1**	**2**	**3**
N	8	8	8
Mean (pg/ml)	67.09	137.15	269.4
Coefficient of Variation (%)	1.18	1.30	6.73

2. **INTER-ASSAY PRECISION**
   To determine between-run precision in Calibrator II, three different samples of known concentration were assayed by replicates on 6 different assays.

Sample	**1**	**2**	**3**
N	6	6	6
Mean (pg/mL)	67.86	138.97	273.05
Coefficient of Variation *(%)*	4.74	2.96	2.17

3. **RECOVERY**

The recovery of mouse IL-17A in mouse serum and cell culture samples was evaluated with mouse IL-17A spiked samples at high, medium and low concentrations.

Serum sample	Expected value	Detected value	Recovery（%）
High	191	192.43	100.74
Medium	108	104.55	96.8
Low	66	71.03	92.9

Cell culture sample	Average Recovery %	Range of Recovery (%)
High	100.43	99.86-101
Medium	98.3	94.3-101.3
Low	98.13	94.5-100.2

4. **SENSITIVITY**

The minimum detectable dose of this mouse IL-17A ELISA kit was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of mouse IL-17A calculated from calibrate I and calibrate II diluted standard curve was <2 pg/mL.

References:

1. Tousif S, Singh Y, Prasad DV, Sharma P. Van Kaer L, Das G. T-cells from Programmaed death-1 deficient mice respond poorly to Mycobacterium tuberculosis infection. PLos One. 2011 May 12;6(5)
2. Interleukin-17 signaling in inflammatory, Kupffer, and hepatic stellate cells exacerbates liver fibrosis in mice. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Osterreicher CH, Stickel F, Ley K, Brenner DA, Kisseleva T. Gastroenterology. 2012 Jun 8.
3. Interleukin-17 deficiency reduced vascular inflammation and development of atherosis in Western diet-induced apo E-deficient mice. Usui F, Kimura H, Ohshiro T, Tatsumi K, Kawashima A, Nishiyama A, Iwakura Y, Ishibashi S, Takahashi M. Biochem Biophys Res Commun. 2012 Mar 30;420(1):72-7
4. The presence of IL-17A and T helper 17 cells in experimental mouse brain tumors and human glioma. Wainwright DA, Sengupta S, Han Y, Ulasov IV, Iesniak. J Clin Invest. 2010 January 4; 120(1): 331–342.