

REFERENCES

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3. Starnes T, et al. (2002). J. Immunol. 169 (2): 642.

Rabbit IL-17A Immunoassay

Catalog Number: SEKRT-0236

For the quantitative determination of Rabbit IL-17A concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

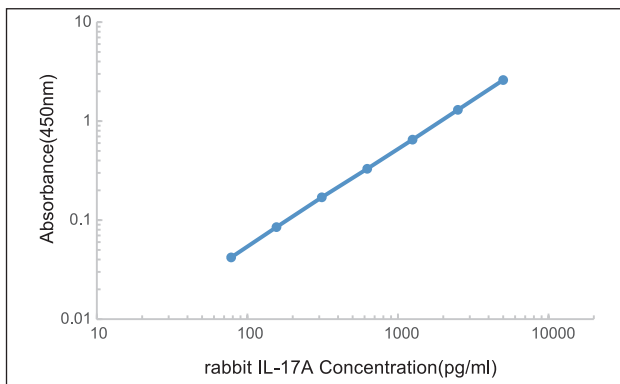
RECOVERY: The recovery of IL-17A spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of IL-17A in two matrices

| Sample Type | Average % of Expected Range (%) | Range (%) |
|---------------------------|---------------------------------|-----------|
| Citrate plasma | 94 | 86-103 |
| Cell culture supernatants | 95 | 86-106 |

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of IL-17A in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

| Dilution ratio | Recovery(%) | Citrate plasma | Cell culture supernatants |
|----------------|----------------------|----------------|---------------------------|
| 1:2 | Average% of Expected | 96 | 106 |
| | Range (%) | 88-105 | 98-117 |
| 1:4 | Average% of Expected | 94 | 107 |
| | Range (%) | 87-106 | 99-119 |



Representative standard curve for IL-17A ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant Rabbit IL-17A. The factors listed below were prepared at 10ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

| Recombinant Rabbit | Recombinant mouse | Recombinant porcine |
|--------------------|-------------------|---------------------|
| TNF- α | IL-2 | |
| IL-2 | IL-17A | |
| IL-4 | IL-4 | |
| IL-6 | | |
| IFN-gamma | | |

BACKGROUND

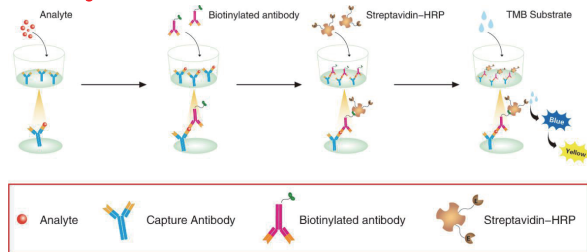
Interleukin 17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation. IL-17 is produced by T-helper cells and is induced by IL-23 which results in destructive tissue damage in delayed-type reactions. Interleukin 17 as a family functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. Interleukin 17 acts synergistically with tumor necrosis factor and interleukin-1. To elicit its functions, IL-17 binds to a type I cell surface receptor called IL17R of which there are at least three variants IL17RA, IL17RB, and IL17RC. The IL-17 family is comprised of at least six proinflammatory cytokines that share a conserved cysteine-knot structure but diverge at the N-terminus. In addition to IL-17A, members of the IL-17 family include IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F. IL-17 family members are glycoproteins secreted as dimers that induce local cytokine production and recruit granulocytes to sites of inflammation. IL-17 is induced by IL-15 and IL-23, mainly in activated CD4+ T cells distinct from Th1 or Th2 cells. IL-17F is the most homologous to IL-17, but is induced only by IL-23 in activated monocytes. IL-17B binds the IL-17B receptor, but not the IL-17 receptor; it is most homologous with IL-17D, which is expressed by resting CD4+ T cells and CD19+ B cells. IL-17E is mainly produced by Th2 cells and recruits eosinophils to lung tissue. IL-17C has a very restricted expression pattern but has been detected in adult prostate and fetal kidney libraries.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-17A has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-17A present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for IL-17A is added to detect the captured IL-17A protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash

to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the IL-17A 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.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the IL-17A ELISA

| Std (pg/mL) | O.D.1 | O.D.2 | Averag | Correct |
|-------------|-------|-------|--------|---------|
| 0 | 0.053 | 0.056 | 0.0545 | --- |
| 93.75 | 0.095 | 0.102 | 0.0985 | 0.044 |
| 187.5 | 0.187 | 0.193 | 0.19 | 0.1355 |
| 375 | 0.296 | 0.304 | 0.3 | 0.2455 |
| 750 | 0.523 | 0.541 | 0.532 | 0.4775 |
| 1500 | 0.965 | 0.942 | 0.9535 | 0.899 |
| 3000 | 1.635 | 1.653 | 1.644 | 1.5895 |
| 6000 | 2.487 | 2.464 | 2.4755 | 2.421 |

DESCRIPTION

with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP(120µL)** - Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 120µL HRP Conjugate sufficient for a 96-well plate. Make 1:100 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 100µL of HRP Conjugate to 10 mL of Streptavidin-HRP Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE.

***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.



Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-Rabbit IL-17A antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 20 minutes at room temperature (25±2°C).



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-20 minutes (depending on signal) at room temperature(25±2°C). Protect from light.



Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

DESCRIPTION

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS & STORAGE CONDITIONS

| PART | SIZE | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|----------|---|
| Microwell Plate - antibody coated 96-well Microplate (8 wells x12 strips) | 1 plate | 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** |
| Standard-lyophilized, 6000pg/ml upon reconstitution | 2 vials | Aliquot and Store at -20°C** for six months |
| lyophilized Biotin-Conjugated antibody | 1 vial | Store at 2-8°C **for six months |
| Concentrated Streptavidin-HRP | 1 vial | Store at 2-8°C **for six months |
| Standard /sample Diluent | 1 bottle | Store at 2-8°C **for six months |
| Biotin-Conjugate antibody Diluent | 1 bottle | Store at 2-8°C **for six months |
| Streptavidin-HRP Diluent | 1 bottle | Store at 2-8°C **for six months |
| 20 x Wash Buffer Concentrate | 1 bottle | Store at 2-8°C **for six months |
| Substrate Solution | 1 bottle | Store at 2-8°C **for six months |
| Stop Solution | 1 bottle | Store at 2-8°C **for six months |
| Plate Cover Seals | 4 pieces | |

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 15 minutes at 1000×g. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

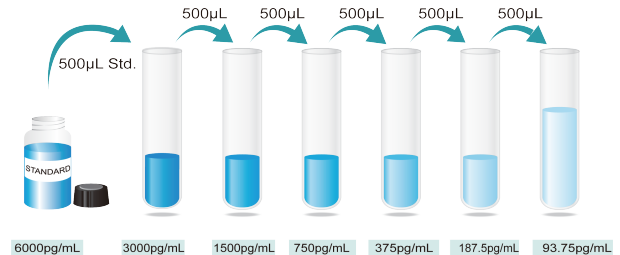
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Note: The normal Rabbit serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25°C) before use.
2. **Wash Buffer** - Dilute 30mL of 20x Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Sample (2 vials)** - Rabbit IL-17A Standard has a total of 2 vials. Each vial contains the standard sufficient for generating a standard curve. Reconstitute the Standard with 1.0mL of **Standard /sample**

Diluent. This reconstitution produces a stock solution of 6000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Sample Diluent into 3000pg/ml tube and the remaining tubes. Use the stock solution of 6000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly (vortex 20 sec for each of dilution step) and change pipette tips between each transfer. The 6000 pg/mL standard serves as the high standard. The Standard/sample Diluent serves as the zero standard (0 pg/mL).

**Preparation of Rabbit IL-17A standard dilutions**

***If you do not run out of re-melting standard, store it at -20°C . Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-Rabbit IL-17A antibody(1 vial)** - The lyophilized Detection Antibody should be stored at 4°C to -20°C in a manual defrost freezer for up to 6 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 110µL of sterile Biotin-Conjugate antibody Diluent to each vial and vortex 30 sec to obtain the stock solution. If the entire 96-well plate is used, take 100µL of detection antibody stock solution into 10 mL of Biotin-Conjugate antibody Diluent to make working dilution of Detection Antibody and mix thoroughly prior to the assay. If the partial antibody is used, make a 1:100 dilution of the concentrated Biotin-Conjugate solution