BioLegend®

Enabling Legendary Discovery™

Rat IL-17A

ELISA MAX™ Deluxe Set

Cat. No. 437904

BioLegend's ELISA MAX™ Deluxe Set contains the components necessary for the quantification of recombinant and natural rat IL-17A in cell culture supernatant, serum, plasma or other biological fluids. The assay is sensitive, accurate, robust and cost-effective.

It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.

Materials Provided

1. Rat IL-17A ELISA MAX™ Capture Antibody (200X)
2. Rat IL-17A ELISA MAX™ Detection Antibody (200X)
3. Rat IL-17A Standard
4. Avidin-HRP (1000X)
5. Substrate Solution A
6. Substrate Solution B
7. Coating Buffer B (5X)
8. Assay Diluent A (5X)
9. Matrix Diluent C (for serum and plasma samples)

Introduction

Rat IL-17A, also known as IL-17, is a member of the IL-17 family of cytokines. IL-17A is mainly produced by Th17 cells and it exhibits multiple biological activities including the induction of various proinflammatory cytokines and chemokines on a variety of cell types. IL-17A has been linked to many immune and autoimmune-related diseases including rheumatoid arthritis, asthma, lupus and multiple sclerosis.

Principle of the Test

BioLegend ELISA MAX™ Deluxe Set contains pre-optimized essential reagents and additional buffers and solutions for Sandwich ELISA assay. A rat IL-17A specific rat monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL-17A binds to the immobilized capture antibody. Next, a biotinylated rat monoclonal anti-rat IL-17A detection antibody is added, producing an antibody-antigen-antibody "sandwich". The Avidin-HRP reagent is subsequently added, followed by TMB Substrate, producing a blue color in proportion to the concentration of IL-17A present in wells. Then Stop Solution should be added to wells to terminate the reaction. This step changes the reaction color from blue to yellow. The absorbance in wells should be read at 450nm using a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H_2SO_4
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution with 1X Assay Diluent A, aliquot the remaining lyophilized standard into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to their appropriate storage conditions.

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. Substrate Solution A and Substrate Solution B are harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze-thaw cycles. Serum specimens should be clear and non-hemolyzed.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze-thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Reagent and Sample Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

NOTE: Precipitation of 5X Assay Diluent A may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.

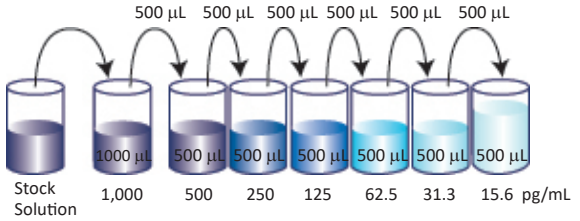
Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard with 0.2 mL of 1X Assay Diluent A by following the instructions described in Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Preparation of 1X Reagent for 1 Plate

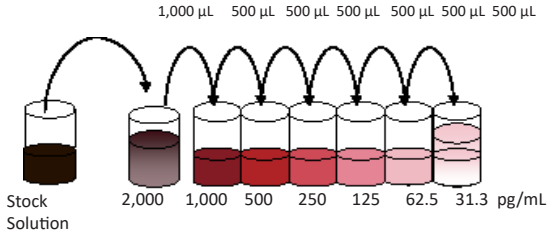
Material	Dilute with
2.4 mL of Coating Buffer A (5X)	9.6 mL of Deionized Water
60 µL of Capture Antibody (200X)	12 mL of 1X Coating Buffer A
12 mL of Assay Diluent A (5X)	48 mL of PBS
60 µL of Detection Antibody (200X)	12 mL of 1X Assay Diluent A
12 µL of Avidin-HRP (1,000X)	12 mL of 1X Assay Diluent A

Prior to use, prepare 1,000 µL of the top standard from the stock solution in 1X Assay Diluent A (**refer to Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol**).

- **For measuring cell culture medium sample:** Prepare 1,000 µL of the top standard at a concentration of 1,000 pg/mL by diluting the stock solution with 1X Assay Diluent A. Perform six two-fold serial dilutions of the 1,000 pg/mL top standard with 1X Assay Diluent A in separate tubes. After diluting, the rat IL-17A standard concentrations are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, and 15.6 pg/mL, respectively. 1X Assay Diluent A serves as the zero standard (0 pg/mL).



- **For measuring serum or plasma samples:** Prepare 1,000 µL of the top standard at a concentration of 2,000 pg/mL by diluting the stock solution with 1X Assay Diluent A. Perform six two-fold serial dilutions of the 2,000 pg/mL top standard with Assay Diluent A in separate tubes. After diluting, the rat IL-17A standard concentrations are 2,000 pg/mL, 1,000 pg/mL 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, and 31.3 pg/mL, respectively. 1X Assay Diluent A serves as the zero standard (0 pg/mL).



TMB Substrate Solution is a mixture of equal volumes of Substrate Solution A with Substrate Solution B. Mix the two components immediately prior to use. For one plate, mix 6 mL of Substrate Solution A with 6 mL of Substrate Solution B in a clean container. The solution should be clear and colorless.

Samples: For measuring cell culture supernatant samples, if dilutions are required, use the control culture medium or 1X Assay Diluent A as the sample diluent.

For measuring serum or plasma samples, a 2-fold dilution of the samples with 1X Assay Diluent A is required. If further dilution is necessary, samples should be further diluted with Matrix Diluent C.

Assay Procedure

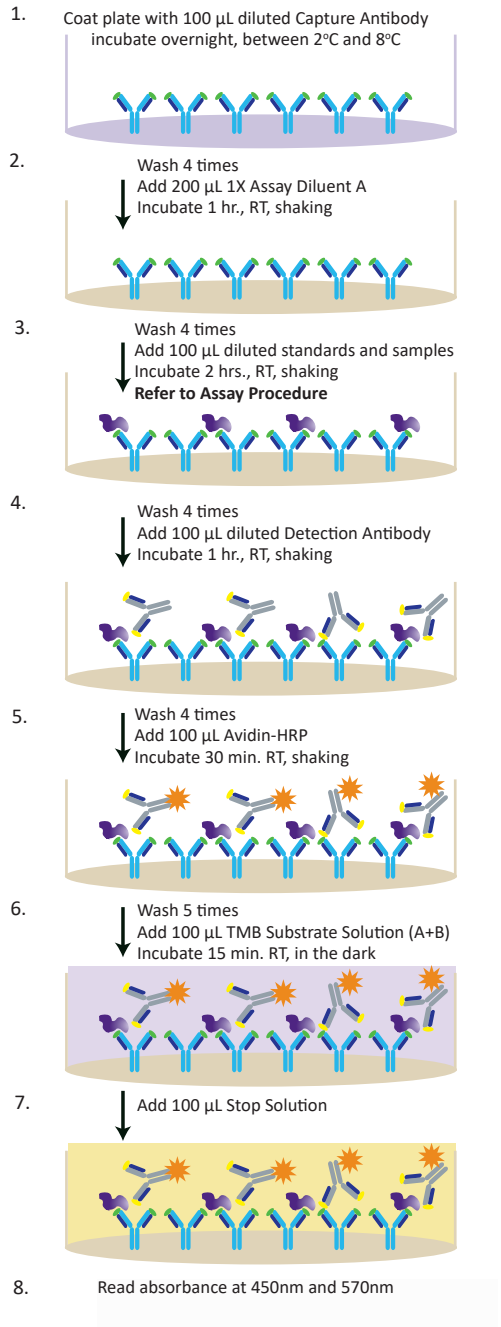
Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. One day prior to running the ELISA, dilute the Capture Antibody with 1X Coating Buffer B as described in step 2 of the Reagent and Sample Preparations section. Add 100 µL of this Capture Antibody solution to all of the wells of the 96-well plate. Seal plate and incubate it overnight (16-18 hrs.) between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Wash the plate 4 times with at least 300 µL of Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL of 1X Assay Diluent A per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.
6. While the plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. **For measuring cell culture medium sample:**
   - Wash plate 4 times with Wash Buffer as in step 3.
   - Add 100 µL/well of the standards and cell culture medium samples to the appropriate wells.

   For measuring serum or plasma samples:
   - Wash plate 4 times with Wash Buffer as in step 3.
   - Add 50 µL/well of Matrix Diluent C to the standard wells. Add 50 µL/well of 1X Assay Diluent A to the sample wells.
   - Add 50 µL/well of the standards prepared above to the standard wells. Add 50 µL/well of the diluted serum or plasma samples to the sample wells.
8. Seal the plate and incubate at RT for 2 hours while shaking.
9. Wash the plate 4 times with Wash Buffer as in step 3.
10. Add 100 µL of the diluted Detection Antibody solution to each well, seal the plate and incubate at RT for 1 hour while shaking.
11. Wash the plate 4 times with Wash Buffer as in step 3.
12. Add 100 µL of the diluted Avidin-HRP solution to each well, seal the plate and incubate at RT for 30 minutes while shaking.
13. Wash the plate 5 times with Wash Buffer as in step 3. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
14. Add 100 µL of freshly mixed TMB Substrate (A+B; in proportion 1:1) and incubate **in the dark** for 15 minutes*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
15. Stop the reaction by adding 100 µL of Stop Solution to each well. Positive wells should turn from blue to yellow.
16. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

Assay Procedure Summary



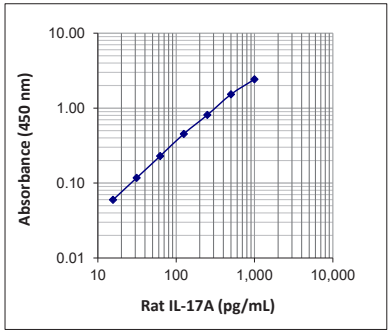
For other technical resources, please visit:
www.biolegend.com/en-us/support or
email: **techserv@biolegend.com**

Calculation of Results

Plot the standard curve on log-log axis graph paper with analyte concentration on the x-axis and absorbance on the y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations in the samples, find the absorbance value of the unknown on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the corresponding analyte concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

Typical Data

Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics

Sensitivity: The expected minimum detectable concentration of IL-17A for this set is 8 pg/mL.

Specificity: This kit cross-reacts with mouse IL-17A. Recombinant mouse IL-17A/F shows approximately 2.2% cross-reactivity in this assay.

No cross reactivity was observed when this kit was used to analyze human IL-17A and the following human, mouse and rat recombinant cytokines/chemokines at up to 50 ng/mL.

IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-15, IL-17B, IL-17F, IL-17E, IL-18, IL-22, IL-23, IL-32α, FGF-basic, GM-CSF, IFN-γ, MCP-1, TNF-α, and TSLP

Troubleshooting

Refer to the troubleshooting guidelines at BioLegend's website for details (http://www.biolegend.com/elisa_troubleshooting).

BioLegend, Inc.

BioLegend is ISO 13485 certified.

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