BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human APRIL/TNFSF13

Cat. No. 439307

ELISA Kit for Accurate Quantitation of Human APRIL/TNFSF13 from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.
biolegend.com

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

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Introduction:

APRIL, or TNFSF13, stands for "A Proliferation-Inducing Ligand", and as its name suggests, it stimulates cell proliferation. It is a member of the TNF superfamily of ligands, being most closely related to BAFF, with which it shares 30% amino acid sequence identity. APRIL is cleaved intracellularly by furin, and is believed to exist mainly as a secreted, soluble form. As with most other TNF family members, APRIL exists as a functional homotrimer (total MW = 90 kD). It can bind to two cell-surface receptors: BCMA and TACI, which it shares with BAFF, to exert downstream T- and B-cell regulatory effects. It also possesses a heparin binding domain, and has been demonstrated to bind to proteoglycans on the cell surface.

APRIL is most well known for its tumor proliferation effects. It is a potential biomarker, with serum levels elevated for certain cancers, and expression levels elevated in cancer tissues. It is also a potential biomarker for certain autoimmune diseases, and in fact, recombinant TACI is currently in clinical trials as a neutralization drug against APRIL and BAFF for the treatment of SLE.

Besides forming homotrimers, APRIL can also form functional heterotrimers with BAFF. The stoichiometric relationship of the protomeric units is still unclear, however it appears that these heterotrimers are significant since they are elevated in the serum of certain autoimmune patients.

APRIL also forms an endogenous chimeric protein with another related protein, TWEAK, effectively called TWE-PRIL. It is believed that TWE-PRIL is membrane bound, and consists of the N-terminus of TWEAK with its transmembrane domain, and the C-terminus of APRIL with its receptor binding domain.

BioLegend's LEGEND MAX™ Human APRIL/TNFSF13 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a monoclonal mouse anti-human APRIL/TNFSF13 capture antibody. The detection antibody is a biotinylated monoclonal mouse anti-human APRIL/TNFSF13 antibody. This kit is specifically designed for the accurate protein quantitation of human APRIL/TNFSF13 from cell culture supernatant, serum, plasma and other biological fluids. It can detect APRIL-BAFF heterotrimers, in addition to APRIL homotrimers. It is analytically validated with ready-to-use reagents.

Tel: 858-768-5800

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-human APRIL/TNFSF13 Pre-coated 96-well Strip Microplate	1 plate		79826
Human APRIL/TNFSF13 Detection Antibody	1 bottle	12 mL	79827
Human APRIL/TNFSF13 Standard	1 vial	lyophilized	79829
Avidin-HRP A	1 bottle	12 mL	79131
Assay Buffer A	1 bottle	25 mL	78232
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP A</td></tr>
<tr><td>Assay Buffer A</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/support/#msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid and is corrosive. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°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 < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human APRIL/TNFSF13 Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX ™ Kit Lot-Specific Certificate of Analysis/LEGEND MAX ™ Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. For measuring serum, plasma, or cell culture supernatant samples, in general, no sample dilution is required. If dilutions are necessary, samples should be diluted with Assay Buffer A.

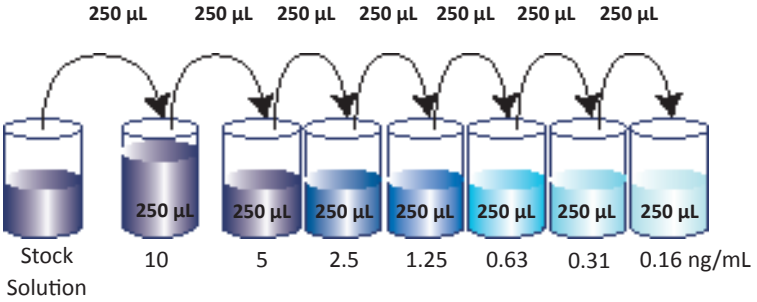
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature 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.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 10 ng/mL top standard by diluting 250 µL of the standard stock solution in 250 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 10 ng/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human APRIL/TNFSF13 standard concentrations in the tubes are 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.31 ng/mL, 0.16 ng/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X 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.

5. Add 50 µL of Assay Buffer A to each well that will contain either standard dilutions or samples.

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.

8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Human APRIL/TNFSF13 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

11. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

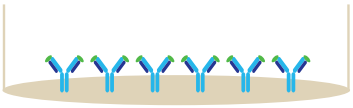
13. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 minutes in the dark. Wells containing human APRIL/TNFSF13 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

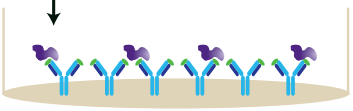
15. Read absorbance at 450 nm immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

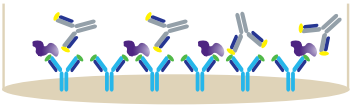
1. Wash 4 times
Add 50 µL Assay Buffer A



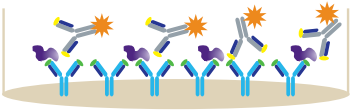
2. Add 50 µL diluted standards or samples
Incubate 2 hrs, RT, shaking



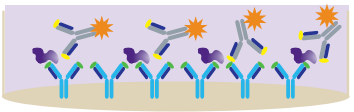
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



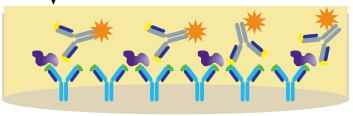
4. Wash 4 times
Add 100 µL Avidin-HRP A solution
Incubate 30 mins, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 15 mins, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

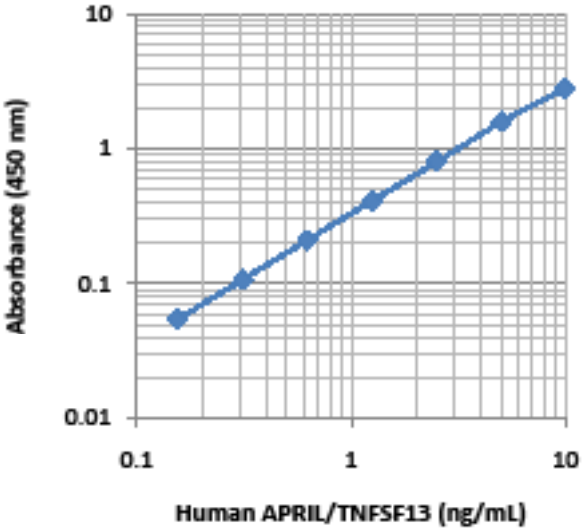
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration 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 cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

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



Performance Characteristics:

Specificity: This kit was used to analyze the following samples with the results summarized in the table below.

Category	Sample	Result
Forms of Human APRIL	APRIL-APRIL-APRIL Homotrimer	Strong reactivity
	APRIL-BAFF-BAFF Heterotrimer	Moderate reactivity
	BAFF-APRIL-APRIL Heterotrimer	Strong reactivity
Closely Related Human Cytokines/ Chemokines each at 50 ng/mL	BAFF, TNF-α, TWEAK, sFasL, CD40L	No or negligible cross-reactivity
Other Human Cytokines/ Chemokines each at 50 ng/mL	IL-1α, IL-1β, IL-1R Antagonist, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-12, IL-12 p40, IL-13, IL-16, IL-17A, IL-17E, IL-17F, IL-17A/F, IL-18, IL-22, IL-23, IL-33, IL-34, CCL20, CCL22, CXCL10, MCP-1, MCP-3, Lipocalin, Nogo-B, GM-CSF, G-CSF, GAPDH, β_2-Microglobulin, VCAM-1, RANTES, TGF-α, Lymphotactin, Eotaxin	No or negligible cross-reactivity
Serum from Other Species	Mouse, Rat, Pig, Horse, Rabbit, Non-human Primate	Strong signal in non-human primate samples. Mild signals in porcine, equine, and rabbit samples. No cross-reactivity with murine or rat samples.
Interference from Soluble Receptor	Effect of recombinant mouse TACI on APRIL ELISA signal	Dose-dependent inhibition
Interference from BAFF	Effect of recombinant human BAFF on APRIL ELISA signal	No inhibition

Sensitivity: The assay sensitivity is approximately 0.038 ng/mL (average MDC of 0.028 ± 0.010 ng/mL in 5 assays).

Recovery: Recombinant human APRIL/TNFSF13, at concentrations of 5, 1.25, and 0.31 ng/mL, was spiked into 8 human serum samples and

2 human plasma samples, then analyzed with the LEGEND MAX™ Human APRIL/TNFSF13 ELISA Kit. On average, 94% of the protein was recovered from the serum samples.

Linearity: 8 human serum samples and 2 human plasma samples with high concentrations of human APRIL/TNFSF13 were diluted with Assay Buffer A to produce samples with values within the dynamic range, and then assayed with the kit to determine the dilution linearity. On average, 107% linearity of dilution was observed.

Intra-Assay Precision: Two samples with different recombinant human APRIL/TNFSF13 concentrations were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (ng/mL)	4.98	0.238
Standard Deviation	0.20	0.008
% CV	4.0	3.4

Inter-Assay Precision: Two samples with different concentrations of recombinant human APRIL/TNFSF13 were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	5	5
Mean Concentration (ng/mL)	4.89	0.254
Standard Deviation	0.07	0.010
% CV	1.4	3.9

Biological Samples:

Serum - Twenty normal human serum samples were assayed for basal levels of human APRIL/TNFSF13. All samples resulted in detectable concentrations, ranging from 0.18-9.4 ng/mL.

Plasma - Twenty normal human plasma samples were assayed for basal levels of human APRIL/TNFSF13. All but two samples resulted in detectable concentrations, ranging from 0.04-5.7 ng/mL.

Cell Culture Supernatant - Human PBMCs were isolated and plated in a 12-well plate at ~2 x 10^6 cells/mL in 10% FBS/RPMI. Cells were stimulated with CD3 + CD28 (2 + 2 µg/mL) or PMA + Ionomycin (20 + 500 ng/mL), and supernatants were removed at one to three days after stimulation and assayed for human APRIL. Unstimulated PBMCs had fairly high levels of human APRIL (~3 ng/mL), but once stimulated, these levels decreased by >50%.

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
8999 BioLegend Way
San Diego, CA 92121
Tel: 1.858.768.5800
Tel US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)
Fax: 1.877.455.9587
Email: info@biolegend.com
biolegend.com

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