INTENDED USE

The Human Anti-Soliris (eculizumab) ELISA Kit is an immunoassay suitable for detecting and quantifying human antibody activity specific for Soliris (Anti-drug Ig's), of any isotype, in human serum or plasma. The assay also detects anti-Soliris antibodies in serum of any other species, including monkey, rat, rabbit and pig.

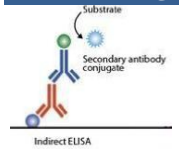
GENERAL INFORMATION

The complement system is a part of the immune system that enhances (complements) the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism. C5a is a protein fragment released from cleavage of complement component C5 by protease C5- convertase into C5a and C5b fragments. C5a is a chemotactic agent and an anaphylatoxin; it is essential in the innate immunity but it is also linked with the adaptive immunity. The increased production of C5a is connected with a number of inflammatory diseases. Eculizumab specifically binds to the terminal Complement component 5, and inhibits the cleavage of C5 to C5a and C5b by the C5 convertase, which prevents the generation of the terminal complement complex C5b-9 (which also has prothrombotic and proinflammatory effects). Both C5a and C5b-9 cause the terminal complement-mediated events that are characteristic of PNH and aHUS.

In people with paroxysmal nocturnal hemoglobinuria (PNH), Soliris improves quality of life and decreases the need for blood transfusions. It is the first approved therapy for paroxysmal nocturnal hemoglobinuria. Eculizumab is also the first agent approved for treatment of atypical hemolytic uremic syndrome (aHUS).

Eculizumab is a recombinant humanized monoclonal IgG2/4 antibody. As with all therapeutic antibodies, there is a potential for immunogenicity with eculizumab. Anti-drug (soliris) antibodies have been observed in 2-3% of PNH and aHUS patient. However, when large amounts of a monoclonal immunoglobulin are continually encountered in circulation, the host may mount significant 'anti-idiotypic' response = anti-Soliris antibodies. Such antibodies might be expected to diminish the effectiveness of Soliris as a drug, and perhaps have other metabolic consequences.

PRINCIPLE OF THE TEST



The Human Anti-Soliris ELISA kit is a double antigen sandwich ELISA based on the binding of anti-Soliris antibodies (any isotype or species) in samples to Soliris antigen immobilized on the microwells; bound anti-Soliris antibody is detected by simultaneously binding to Soliris conjugated to HRP. After a washing step, chromogenic substrate (TMB) is added and color is developed, which is directly proportional to the amount of antiSoliris antibody present in the sample. Stopping Solution is added to terminate the reaction, and A450nm is measured using an ELISA reader.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume10ml+990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume,10ml+190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample/Conjugate Diluent** and store at 2-8°C until the kit lot expires or is used up..
Soliris- HRP Conjugate Concentrate (100x) Part: 210-414, 0.15ml	Peroxidase conjugated Soliris in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Soliris Microwell Strip Plate	210-411	8-well strips (12)	Coated with soliris, (eculizumab) And post-coated with stabilizers.
Anti-Soliris Calibrators			
1U/ml	210-412B	0.65 ml	Four (4) vials, each containing anti-Soliris antibodies; in buffer with protein, detergents and antimicrobial as stabilizers
3U/ml	210-412C	0.65 ml	
6U/ml	210-412D	0.65 ml	
15U/ml	210-412E	0.65 ml	
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10m A multichannel pipettor is recommended
- Disposable glass or plastic 5-15ml tubes for diluting samples and Soliris HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength..

LIMITATIONS OF THE ASSAY

Quantitation of Antibody in a Sample

The ELISA measures anti-Soliris activity, a combination of antibody concentration and avidity for the Soliris antigen. Antibodies with substantially different total Ig concentrations may display similar anti-Soliris activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of Ig (e.g., ug/ml).

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Antibody Stability

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen.

Assay Design

Review Calculation of Results (p6-7) and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than that of the 1 U/ml Calibrator. This is usually 1/50 or greater dilution for human sera with normal levels of IgG and IgM.
- Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of Calibrators. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve, Method A, has limitations. See Limits of the Assay (above).

Plate Set-up:

Bring all reagents to room temperature (18-30°C) quilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8Calibrator wells and 2 wells for each sample and internal control to be assayed.

- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

ASSAY DESIGN AND SET-UP (Continued)

Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

ASSAY PROCEDURE

All steps are performed at room temperature. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation (100ul - 60 min; 4 washes)

* Add 100ul of calibrators, samples and controls each to pre-determined wells.
* Tap the plate gently to mix reagents and incubate for 60 minutes.
* Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation (100ul - 30 min; 5 washes)

* Add 100ul of diluted Soliris HRP to each well.
* Incubate for 30 minutes.
* Wash wells 5 times as in step 2.

3. Substrate Incubation (100ul - 15 min]

* Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
* Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop step (Stop: 100ul)

* Add 100ul of Stop Solution to each well.
* Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. **Absorbance Reading**

* Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
* Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

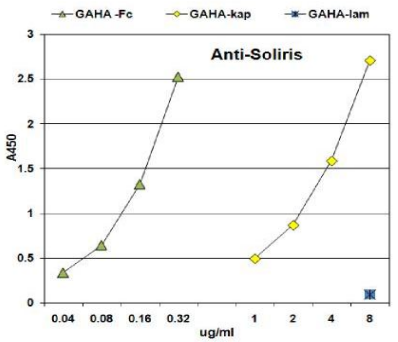
ASSAY PERFORMANCE

Detection Range and Specificity
The Antigen Sandwich ELISA format allows for the detection and quantitation of 'bridging' bivalent and/or multi-valent antibodies of any animal species or of any immunoglobulin isotype and/or subclass – IgG, IgM, IgA or IgE.
This graph shows dilution curves of affinity-purified antibodies reactive with Soliris as antigen, as follows:

GAHA-Fc – goat polyclonal antibodies specific for the Fc region of Soliris; affinity-purified.

GAHA-kap – goat polyclonal antibodies specific for the kappa light chain of Soliris; affinity-purified.

GAHA-lam – goat polyclonal antibodies specific for human lambda light chain; Soliris has no lambda light chain.



Results-

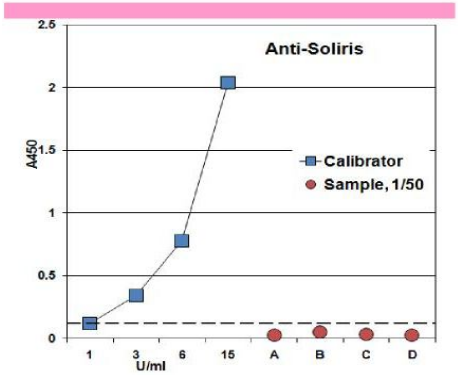
- The data demonstrate measuring antibodies of different specificities.
- This assay, as with all other assays that measure antibody activity, produces a) different signal levels with equivalent amounts of each antibody, or b) the same signal level with different amounts of each antibody. This means that an individual antibody, calibrated in mass units (e.g., ng/ml) cannot serve as a standard curve to quantify other antibodies in mass units.
- The values for the GAHA-kap antibody were not consistentwhen read from different regions of the GAHA-Fc curve – a measure of non-parallelism. When parallelism does not occur, e.g., when antibodies differ significantly in avidity for the Soliris as antigen, use a different method for quantitation (e.g., Method B or C, page 6,7).

INTERPRETATION OF RESULTS

Calculation of Results
Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine Positive Immune and Negative Non-immune or Pre-immune, and to Quantitate positive antibody levels.

Method A. Use of a Calibrator Curve
When the dilution curves of samples are parallel to the Calibrator curve (see Limits of the Assay, page 4, and Assay Performance, page 6), the anti-Soliris activity units may be determined by interpolation from the Calibrator curve.
Sample values= curve value, U/ml x 1/sample dilution

Method B. **Antibody Activity Threshold Index**
Compare Samples to **1 U/ml Calibrator or Internal Control = Positive/Negative Cut-off.**



Results
The sensitivity of the assay to detect anti-Soliris, native level or from drug administration, is controlled so that the 1 U/ml Calibrator represents a threshold OD for most true positives in human serum diluted in the Sample Diluent at 1:50 or greater.
The **1 U/ml Calibrator** can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative, as follows:
* Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 is **Negative** for antibody.

This calculation also quantifies the **positive** Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

INTERPRETATION OF RESULTS (Cont)

C. Positive Index: Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by **calculation of a Positive Index**. One typical method is as follows:

- Calculate the net OD mean + 2 SD of the Control/Non¬immune samples = Positive Index.

- Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 is Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.
This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

PRODUCT SPECIFICATIONS

Specificity

Purified Soliris (eculizumab) is used to coat the microwells; thus the assay is specific for antibodies directed to Soliris or other similar human IgG. The Soliris HRP conjugate reacts with divalent or multivalent antibodies of any isotype (IgG, IgM, IgA, IgE) that are specific to Soliris, and have bound to the Soliris on the plate. Anti-Soliris antibodies from any species may be detected in the assay.
Assay Sensitivity

The Soliris antigen coating level and HRP conjugate concentration are optimized to differentiate anti-Soliris from background (non-antibody) signal with human serum samples diluted 1:50.

PRECAUTIONS AND SAFETY

Controls, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND.

Technical Support available

Toll Free at 1800-3000-8822

Human Anti-Soliris (Eculizumab) antibodies (ADC) ELISA Kit

For Quantitation of Anti-Soliris Antibodies (ADA) in Serum, Plasma or other Biological Fluids

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INSTRUCTION MANUAL

Cat No. ME-210-410-AEG (96 TESTS)

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