INTENDED USE

The Soliris (Eculizumab) ELISA Kit is an immunoassay for quantifying Soliris activity in serum or plasma, or in other appropriately qualified samples from cell culture, bioprocessing solutions, or tissue fluids (e.g., saliva, mucosa). The assay has been specifically validated for quantifying Soliris in human serum or purified drug in biological buffers. It may be used in other species such as mouse and rat.

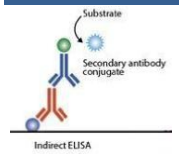
GENERAL INFORMATION

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 Soliris ELISA kit is based on the binding of Soliris in samples to C5 coated on the plates. Bound Soliris is detected with anti-Soliris IgG-HRP conjugate. After a washing step, TMB substrate is added and color (blue) is developed. Stopping Solution is added to terminate the reaction, and color (yellow) A450nm is then measured using an ELISA reader. The concentration of Soliris in samples and control is calculated from Soliris standards.

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
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 Diluent** and store at 2-8°C until the kit lot expires or is used up
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 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.
Anti-Human IgG - HRP Conjugate Concentrate (100x) Part No. 1754, 0.15ml	in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample 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
C5 coated Microwell Strip Plate	210-401	8-well strips (12)	Coated with humanC5, and post-coated with stabilizers.
Soliris Standards			
5 ng/ml 10 ng/ml 25 ng/ml 50 ng/ml 100 ng/ml	210-403B 210-403C 210-403D 210-403E 210-403F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Provided in in buffer with protein, detergents and non-azide antimicrobials as stabilizers.
Positive Control [Soliris] range on label	210-402	0.65 ml	In buffer with protein, detergents and non-azide antimicrobials as stabilizers.
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 1-10ml. A multichannel pipettor is recommended.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.

- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% 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 can be requested or obtained from the Arsh Biotech website.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Culture medium, bioprocessing preparations, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 8). For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For all samples, clarify 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.

Assay Validation

Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism (see Limits of the Assay, page 8), as follows:

Recovery – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the Soliris sample relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions a minimum 5-fold sample dilution is usually sufficient. Serum and plasma require at least a 1/100 dilution to obtain consistent quantitation or complete antigen recovery (see graph on page 6).

Parallelism – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

Plate Set-up

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

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and 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.
- Add 200-310ul 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 Anti-Human IgG HRP Conjugate 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.

CALCULATION OF RESULTS

1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, Soliris concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis)
   against the concentration (ng/ml) of Soliris (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The Soliris concentrations in unknown samples and controls
   can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 100 ng/ml standard should be further diluted and re-assayed.

Typical Results

Wells	Calibrators & samples	A_{450}	Ng/ml
A1, A2	Diluent Blank	0.03	0
B1, B2	5 ng/ml **Standard**	0.31	5
C1, C2	10 ng/ml **Standard**	0.55	10
D1, D2	25 ng/ml **Standard**	0.98	25
E1, E2	50 ng/ml **Standard**	1.35	50
F1, F2	100 ng/ml **Standard**	1.87	100
G1, G2	**Positive Control** [21-37 ng/ml]	1.10	31
H1, H2	Sample [Diluted 1:100]	1.41	56

Calculated: 100-fold dilution x 56 ng/ml = **5.6** ug/ml in serum

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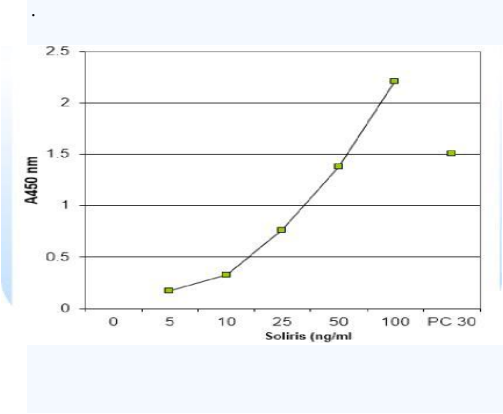


PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit are specific for Soliris and normal human IgG, and do not react with IgM, IgA or IgE, (see Serum: Recovery and Parallelism). Since the Soliris ELISA is based upon the binding of Soliris to the plates, the host specific IgG (human, mouse or rat) will not bind to the coated plates. Therefore, this test is independent of the species. The test has been validated for human; non-human samples (e.g., mouse) can also be used This ELISA may be used for other appropriately qualified species such as rat, mouse, and monkey etc.

Precision

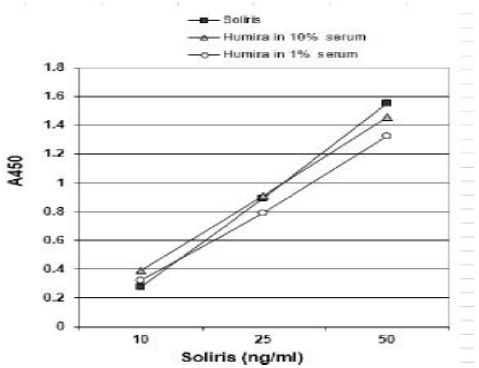
Samples containing low, medium and high concentrations of Soliris were assayed as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

Soliris concentrations were measured with goodbetween assay (3.3 to 6.8 %CV) reproducibility.

Sample	Soliris ng/ml	Inter-assay %CV
High Concn	59.6	**3.3**
Medium Concn	22.5	**6.8**
Low Concn	9.8	**4.5**

Recovery and Parallelism

Soliris was diluted at 4 concentrations into Sample Diluent containing 1% and 10% human serum , and assayed in duplicate. Dilution curves are shown in the following graph



Soliris in 1% serum was quantified essentially equivalently to Soliris in Sample Diluent (Standard Curve). Quantitation was somewhat depressed in 10% serum (1/10 dilution). Therefore dilute samples 1/100 or more for accurate quantitation.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an Soliris concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Sample Diluent blank should also be run; OD should be <0.3 and lower than 1 ng/ml Standard OD.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. Do not rely on results generated from an assay with these issues.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

STORAGE AND STABILITY

The ELISA 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.

LIMITS OF THE ASSAY

1. The The **recovery,** or accuracy of Soliris measurement in human serum (pooled), appears unaffected when diluted at least 1/100 (1%) in Sample Diluent. Recovery in fresh, individual serum or plasma samples has not been determined.
2. A population PK analysis with a standard 1-compartmental model in 40 PNH patients receiving the recommended Soliris regimen, the clearance of Soliris for a typical PNH patient weighing 70 kg was 22 mL/hr and the volume of distribution was 7.7 L. The half-life was 272 + 82 hrs. The mean observed peak and trough serum concentrations of Soliris by week 26 were 194 + 76 mcg/mL and 97 + 60 mcg/mL, respectively. (see Soliris manaual).
3. Lower limits of Soliris detection is ~1.5 ng/ml.

Technical Support available

Toll Free at 1800-3000-8822

Soliris/Eculizumab ELISA Kit

For Quantitation of Soliris (Anti-C5 Activity) in Human Serum or plasma or other biological fluids

INSTRUCTION MANUAL

Cat No. ME-210-400-EHG (96 TESTS)

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