

Anti-SLA (Soluble Liver Antigen) ELISA

Catalog Number:
SLA31-K01

Enzyme immunoassay for the determination of
IgG antibodies to the soluble liver antigen
in human serum and plasma



20A Northwest Blvd., Suite 112,
Nashua, NH 03060
Phone: 617-419-2019
Fax: 617-419-1110
www.EagleBio.com

*For Research Use Only (RUO). Not for use in clinical, diagnostic or
therapeutic procedures.
v. 1.0*

The Eagle Biosciences Anti-SLA ELISA Assay Kit is used for the quantitative determination of IgG antibodies to soluble liver antigen (SLA) in human serum or plasma for the diagnosis of autoimmune hepatitis (AIH).

The group of primary autoimmune liver disease (PAL) comprises AIH, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

The clinical picture of PAL is in most cases not different from other chronic liver diseases. About 15% of all cases with chronic liver diseases show an autoimmune pathogenesis. Therefore, after exclusion of infectious aetiology especially by viruses, the determination of different autoantibodies is recommended.

Patients suffering from AIH show a variety of autoantibodies. Due to the appearance of different antibody specificities classification of AIH into different subgroups is discussed. Type I is characterized by the occurrence of antinuclear antibodies (ANA) and antibodies to smooth muscles (ASMA). ASMA recognize antigenic structure formed by polymeric f-Actin. For type II a high prevalence of antibodies to liver and kidney microsomal antigens (LKM) has been described. LKM1 antibodies recognize epitopes of cytochrome P450 IID6, a 50 kDa cytoplasmic protein found in hepatocytes and proximal tubular kidney cells. LC1 antibodies are specific for type II hepatitis, too. The respective antigen is formiminotransferase / cyclodeaminase located in the cytosol of liver cells. Patients with type III autoimmune hepatitis exhibit antibodies to the soluble liver antigen (SLA) and to the liver pancreas antigen (LP). It has been shown that both antibody species recognise the same antigen and thus are identical. The yet unknown antigen is suggested to be an UGA suppressor tRNA-associated protein. However, type III AIH is not yet fully accepted as independent subgroup for autoimmune hepatitis by the "International Hepatitis Group".

SLA is found in up to 20% of AIH cases and very often the only autoantibody associated with AIH to be detected.

Czaja AL. Natural history, clinical features and treatment of autoimmune hepatitis. *Semin Liver Dis* (1987) 4: 1 – 12

Johnson PJ, McFarlane IA. Meeting Report: International Autoimmune Hepatitis Group. *Hepatology* (1993) 18: 998 – 1005

Wies I, Brunner S, Henninger J, Herkel J, Kanzler S, Meyer zum Büschenfelde KH, Lohse AW: Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. *Lancet* 355: 1510-1515

PRINCIPLE OF THE TEST

The Eagle Biosciences Anti-SLA ELISA Assay Kit is an enzyme immunoassay for the quantitative determination of IgG antibodies to SLA.

The antibodies of the calibrators, controls, and diluted patient samples react with SLA immobilized on the solid phase of microtiter plates. The use of human recombinant SLA guarantees the specific binding of SLA antibodies of the specimen under investigation. Following an incubation period of 30 min at room temperature (RT), unbound serum components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at RT. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (HCl) into the wells after 30 min at RT turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, haemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires -20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	Microtiter plate , 12 breakable strips per 8 wells coated with human recombinant SLA	1 vacuum sealed with desiccant
B BUF WASH 50x	Concentrated wash buffer sufficient for 1000 ml solution	20 ml concentrate capped white
C DIL 5x	Concentrated sample diluent	20 ml concentrate capped white
D CONJ	Conjugate containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped blue
E SOLN TMB	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped black
F HCl 1.0 M	Stop solution 1.0 M hydrochloric acid	15 ml ready for use capped white
1 - 6 CAL	Calibrators (diluted serum) conc.: 0, 3, 10, 30, 100, 300 U/ml	1.5 ml each ready for use
P CONTROL	Positive control (diluted serum) conc.: see leaflet enclosed	+ 1.5 ml ready for use capped red
N CONTROL	Negative control (diluted serum) conc.: see leaflet enclosed	- 1.5 ml ready for use capped green

Materials required in addition

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- glassware
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

Anti-SLA has been designed for 96 determinations

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-SLA have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 50 times with de-ionized or distilled water. For example, dilute 1 ml of the concentrate with 49 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Prepare a sufficient amount of sample diluent by diluting the concentrated diluent 5 times with de-ionized or distilled water. For example, dilute 10 ml of the concentrate with 40 ml of distilled water. The sample diluent prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- Dilute patient sera with sample diluent (made of C) 1+100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (made of C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
100 µl calibrators (1 - 6)
100 µl positive (P) and negative (N) control
100 µl diluted patient samples
into the respective wells.
3. Incubate **30 min** at room temperature (18-25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) to each well.
6. Incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **30 min protected from light** at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 6 on the ordinate, y-axis, (lin. scale) versus their respective Anti-SLA IgG concentrations on the abscissa, x-axis, (log. scale).

SLA antibody concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

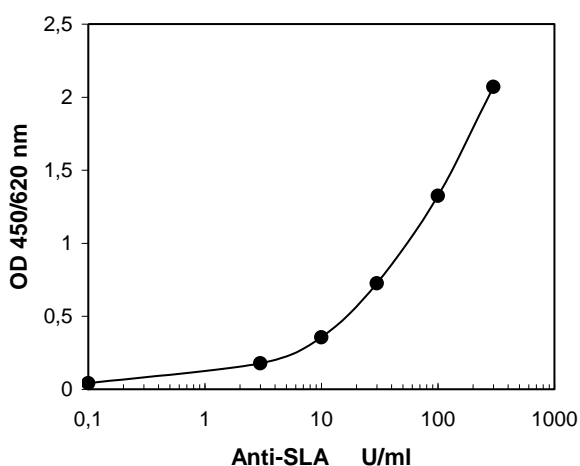
Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Anti-SLA may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.

Example of Typical Assay Results

Well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator 1	0.043	0.039	0.041	0
Calibrator 2	0.184	0.172	0.178	3
Calibrator 3	0.363	0.349	0.356	10
Calibrator 4	0.734	0.716	0.725	30
Calibrator 5	1.440	1.410	1.425	100
Calibrator 6	2.109	2.031	2.070	300
Patient 1	0.658	0.644	0.651	26

TYPICAL STANDARD CURVE



Specimens with an OD > calibrator 6, should be diluted with SLA antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.

Do not use this example for interpreting results.

Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.15
- the mean OD of the calibrator 6 is ≥ 1.3

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution,

wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Anti-SLA	U/ml
positive	> 18
borderline	12 – 18
negative	< 12

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-SLA levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-SLA. However, SLA autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

No international reference material for this parameter is available thus the assay is calibrated in arbitrary units.

Linearity

Selected positive serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be sera that do not follow this rule.

Analytical Sensitivity

The analytical sensitivity of this assay was determined at 1.0 U/ml.

Specificity and Sensitivity

No cross reactivity to other autoantigens have been found. Anti-SLA exhibits a diagnostic specificity of

100%. The diagnostic sensitivity of the assay is 30% in patients suffering from autoimmune hepatitis.

Precision

Intraassay variability

sample	mean U/ml	standard deviation (%)	CV (%)
1	160.0	3.8	2.4
2	85.0	1.9	2.8
3	20.8	0.65	3.1

Interassay variability

sample	mean U/ml	standard deviation (%)	CV (%)
1	154.0	2.8	1.8
2	80.0	2.2	2.8
3	18.4	0.63	3.4

Anti-SLA (Soluble Liver Antigen) ELISA Assay Kit

ASSAY SCHEME

Dilute patients sample 10 µl serum + 1.0 ml sample diluent (made of C)

1	Bring all ready for use reagents to room temperature (18-25°C) before use.				
2	Pipette	Calibrators (1 - 6) Control (P + N) prediluted 1 + 100 patient sera	100 µl	100 µl	100 µl
3	Incubate 30 minutes at room temperature (18-25°C)				
4	Wash Decant, 3 x 300 µl (made of B)				
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate 30 minutes at at room temperature (18-25°C)				
7	Wash Decant, 3 x 300 µl (made of B)				
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light 30 minutes at room temperature (18-25°C)				
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.				

SAFETY PRECAUTIONS

- **This Eagle Biosciences Anti-SLA ELISA Assay Kit is for research use only.** Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

- Since the Anti-SLA ELISA Assay kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.