Anti-ASGPR (Asialo-Glycoprotein Receptor) ELISA Assay Kit

Catalog Number:
AAS31-K01

Enzyme immunoassay for the determination of IgG antibodies to asialoglycoprotein receptor in human serum or plasma

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v. 1.0

INTENDED USE

Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit is used for the semi-quantitative determination of IgG antibodies to asialoglycoprotein receptor (ASGPR) in human serum or plasma.

ASGPR is a liver specific membrane receptor playing a pivotal role in the endocytosis of glycoproteins from the blood. An induction of humoral and cellular immune mechanisms to the ASGPR has been observed in the course of inflammatory liver disorders especially autoimmune hepatitis. The level of ASGPR autoantibodies correlates with the severity of the disease and declines under therapy. The group of primary autoimmune liver disease (PAL) comprises autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

Autoimmune hepatitis is a chronic inflammation of the liver with a yet unknown etiology. It comprises mild clinical forms as well as severe progressive hepatitis with lethal outcome. Females are more frequently affected. Clinical signs of the disease can occur as early as in their twenties.

PRINCIPLE of the TEST

Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay is an enzyme immunoassay for the semi-quantitative determination of IgG antibodies to ASGPR.

The antibodies of the calibrators and diluted samples react with ASGPR immobilized on the solid phase of microtiter plates. ASGPR highly purified from rabbit liver and coated on the microtiter plate guarantees the specific binding of ASGPR IgG antibodies of the specimen under investigation. Following an incubation period of 60 min at 37 °C, unbound serum components are removed by a washing step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP) within an incubation period of 30 min at 37 °C. Excessive conjugate is separated from the solid-phase immune complexes by the following washing step.

Horseradish peroxidase 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 (H_2SO_4) into the wells after 10 min at room temperature 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.

SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, haemolytic and contaminated samples should not be used.

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.

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

TEST COMPONENTS for 96 determinations

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with ASGPR (rabbit)	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG-(sheep) coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
P CONTROL	**Positive control** (diluted serum) +	1 ml ready for use
Co CONTROL	**Cut-off control** (diluted serum) C	1 ml ready for use
N CONTROL	**Negative control** (diluted serum) –	1 ml ready for use

Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
  trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- incubator (37 °C)
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

Size and storage

Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay kit on the box labels.

Upon receipt, all components of the Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay 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 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution 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 sample sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature before use. Mix gently without causing foam.
2. Dispense
   100 µl controls (P, Co, N)
   100 µl diluted patient samples
   into the respective wells.
3. Seal plate, incubate **60 min** at 37 °C.
4. Decant, then wash each well **five** times using **300 µl** wash buffer (B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate, incubate **30 min** at 37 °C.
7. Decant, then wash each well **five** times using **300 µl** wash buffer (B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **10 min protected from light** at room temperature.
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the optical density at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

Results are interpreted by calculating the following ratio of OD:

ratio = $OD_{sample}/OD_{cut\text{-}off\ control}$

This calculation can be done by the integrated evaluation software of the microplate reader used, too.

Example of typical assay results

wells	OD (a)	OD (b)	OD (mean)	ratio
Positive control	1.472	1.450	1.461	3.8
Cut-off control	0.387	0.373	0.380	1.0
Negative control	0.022	0.018	0.020	0.1
Patient 1	0.144	0.134	0.149	0,4 negative
Patient 2	0.591	0.559	0.575	1,5 positive
Patient 3	0.183	0.190	0.186	0,5 negative

Test validity

The test run is valid if:

- the mean OD of the negative control is ≤ 0.3
- the mean OD of the positive control is ≥ 0.7

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-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit	ratio
negative	**< 0.9**
grey zone	**0.9 – 1.1**
positive	**≥ 1.1**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit levels. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

CHARACTERISTIC ASSAY DATA

Calibration

Due to the lack of an international reference material the content of ASGPR autoantibodies in samples is given as ratio of OD.

Linearity

Defined dilutions of the reference material with Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit free human serum are found congruent to calculation with Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit.

Sensitivity

The analytical sensitivity of the Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit was determined at 0.3.

Specificity

The high quality of the immobilized ASGPR ensures the exclusive reaction of ASGPR autoantibodies.

Functional assay sensitivity

This functional assay sensitivity generally represents that concentration which corresponds to the 10 % (intraassay) and to the 20 % (interassay) coefficient of variation in the respective precision profiles of the assay in the lower concentration range. Upon correct and thorough performance of Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit, this value is found at a ratio of 0.4.

Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit values below this defined level of functional assay sensitivity do not meet the statistical criteria for reliability according to GLP (Good Laboratory Practice) and therefore can not be distinguished from zero due to the statistically necessary certainty.

Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit concentrations above a ratio of 0.4, however, fulfil these criteria and are consequently assessed as valid.

Precision

Intraassay		Interassay	
mean (ratio)	CV %	mean (ratio)	CV %
0.5	6.6	0.4	16.2
1.4	2.8	1.2	10.8
2.8	6.5	4.2	7.5

ASSAY SCHEME

Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit

Dilute sample	**10 µl serum + 1.0 ml sample diluent (C)**

1	**Bring all reagents to room temperature (18-25°C)**			
2	Pipette	controls (P, Co, N) 1 + 100 prediluted sera	100 µl	100 µl
3	Seal plate and incubate		60 min, 37 °C	
4	Wash		Decant, 5 x 300 µl (made of B)	
5	Pipette conjugate (D)		100 µl	100 µl
6	Seal plate and incubate		30 min, 37 °C	
7	Wash		Decant, 5 x 300 µl (made of B)	
8	Pipette substrate (E)		100 µl	100 µl
9	Incubate protected from light		10 min, room temperature (18-25°C)	
10	Pipette stop solution (F)		100 µl	100 µl
11	Read at 450 nm against 620 (690) nm within 30 min.			

SAFETY PRECAUTIONS

- **This Anti-ASGPR (Asialoglycoprotein Receptor) ELISA Assay Kit is for research use only**. Follow the working instructions carefully. GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. 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 preservatives. 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 for HIV as well as 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-ASGPR (Asialoglycoprotein Receptor) 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.

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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.