INSTRUCTION MANUAL

REF **3900**

August 24, 2015

Anti-ASGPR

- 96 determinations -

CE

IVD *In vitro* diagnostic device

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

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction	D	Biological risk

GA GENERIC ASSAYS GmbH

Ludwig-Erhard-Ring 3

15827 Dahlewitz, Germany

Telephone: +49 (0) 33708-9286-0
Fax: +49 (0) 33708-9286-50

www.genericassays.com

INTENDED USE

Anti-ASGPR 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.

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). For type II a high prevalence of antibodies to liver and kidney microsomal antigens (LKM) has been described. LC1 antibodies are specific for type II hepatitis, too. Patients with type III autoimmune hepatitis exhibit antibodies to the soluble liver antigen (SLA).

ASGPR autoantibodies can be detected in sera of up to 76% of patients suffering from AIH. However, patients with viral hepatitis may develop ASPGR autoantibodies, too. Therefore a viral genesis of the liver disorder should be excluded.
Determination of autoantibodies to ASGPR supports the follow-up and the differential diagnosis of toxic and other chronic inflammatory liver disorders.

McFarlane BM, McSorley CG, Vergani D, McFarlane IG, Williams R: Serum autoantibodies reacting with the hepatic asialoglycoprotein receptor protein (hepatic lectin) in acute and chronic liver disorders. J Hepatol 1986 3, 196-205

Treichel U, Poralla T., Hess G, Manns M, Meyer zum Buschenfelde KH: Autoantibodies to human asialoglycoprotein receptor in autoimmune-type chronic hepatitis. Hepatology 1990 11, 606-612

PRINCIPLE of the TEST

Anti-ASGPR is an enzyme immunoassay for the semi-quantitative determination of IgG antibodies to ASGPR.

The antibodies of the calibrators and diluted patient 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 room temperature, 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 room temperature. 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 15 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.

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 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.

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 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-(goat) 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
- 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 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-ASGPR 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 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 patient 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 RT (18-25 °C).
4. Decant, then wash each well **three** 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 RT (18-25 °C).
7. Decant, then wash each well **three** times using **300 µl** wash buffer (B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 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	1.657	1.710	1.684	5.3
Cut-off control	0.321	0.309	0.315	1.0
Negative control	0.111	0.108	0.110	0.4
Patient 1	0.144	0.134	0.149	0.5 negative
Patient 2	0.591	0.559	0.575	1.8 positive
Patient 3	0.183	0.190	0.186	0.6 negative

Test validity

The test run is valid if:

- the mean OD of the positive control is ≥ 0.6
- the mean OD of the cut-off control > mean OD of the negative control

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	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 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-ASGPR. However, ASGPR 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

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

Linearity

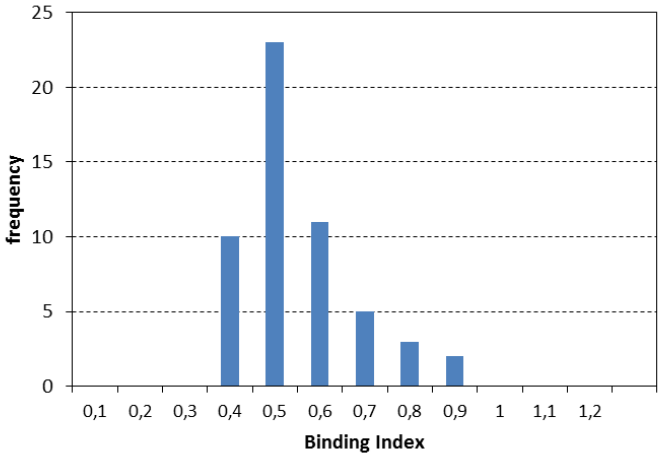
Defined dilutions of the reference material with Anti-ASGPR free human serum are found congruent to calculation with Anti-ASGPR.

Sensitivity

The analytical sensitivity of the Anti-ASGPR was determined at 0.3.

Specificity

Frequency distribution of antibodies in Anti-ASGPR. 54 unselected human sera were tested. All sera were found negative. This corresponds to a diagnostic specificity of 100%.



Precision

Intraassay (n=20)		Interassay (10x5)	
mean (ratio)	CV %	mean (ratio)	CV %
1.5	5.0	1.7	9.1
3.5	7.4	3.9	8.1
7.2	5.5	7.9	8.4

INCUBATION SCHEME

Anti-ASGPR (3900)

Dilute patients 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, RT (18-25 °C)	
4	Wash		Decant, 3 x 300 µl (made of B)	
5	Pipette conjugate (D)		100 µl	100 µl
6	Seal plate and incubate		30 min, RT (18-25 °C)	
7	Wash		Decant, 3 x 300 µl (made of B)	
8	Pipette substrate (E)		100 µl	100 µl
9	Incubate protected from light		15 min, RT (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 kit is for in vitro 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 Neolone® M10 (< 0.1 % w/w) 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 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.