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Instruction For Use
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ORGENTEC

CE

US Market: For Research Use Only

ORG 550   Anti-GBM

NAME AND INTENDED USE

Anti-GBM is an ELISA test system for the quantitative measurement of IgG class autoantibodies to glomerular basement membrane (GBM) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device	MICROPLATE		Microplate
	Manufacturer	CALIBRATOR A		Calibrator
REF	Catalogue number	CALIBRATOR B		Calibrator
		CALIBRATOR C		Calibrator
∑ 96	Sufficient for 96 determinations	CALIBRATOR D		Calibrator
		CALIBRATOR E		Calibrator
LOT	Batch code			
	Use by	CONTROL +		Control positive
		CONTROL −		Control negative
	Temperature limitation			
	Consult instructions for use	DILUENT		Sample Buffer P
		CONJUGATE		Enzyme Conjugate
	Keep away from sunlight			
		TMB		TMB Substrate
	Do not reuse	WASH		Stop solution
	Date of manufacture	STOP		Wash Buffer
CE	conform to European directive 98/79/EC	RTU		Ready to use

PRINCIPLE OF THE TEST

Highly purified GBM is bound to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stopps the reaction generating a yellow end-product. The intensity of the yellow color

correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

SUMMARY AND EXPLANATION OF THE TEST

Primarily, Goodpasture syndrome is an autoimmune disorder of the kidneys. Ernest Goodpasture, an American pathologist was the first in 1919 who described the coexistence of a fatal lung hemorrhage coming along with proliferative glomerulonephritis in a young man. The syndrome is now considered as an autoimmune disorder consisting of the triad of glomerulonephritis, lung hemorrhage and anti-glomerular basement antibodies formation.

The incidence of the Goodpasture syndrome is about 0.5 to 1 cases per million inhabitants per year. Patients in the third and seventh decade are mainly affected. Goodpasture syndrome is a medical emergency with a case fatality rate of 75 to 90 % due to kidney and respiratory insufficiency, if not treated. Histological, the disorder is characterized by continuous linear deposition of immunoglobulins along the glomerular basement membrane (GBM), demonstrable by direct immunofluorescence on kidney biopsies. Nowadays, the determination of circulating autoantibodies against the C-terminal end of the α-3 chain of type IV collagen is considered the diagnostic criterion. Basement membranes form an anatomical barrier wherever epithelia meet connective tissue. Type IV collagen that is only found in GBM forms a matrix in which additional molecules (e.g. Laminin, Entactin) are integrated. Three out of six alpha-chains (polypeptides with more than 1,650 amino acids) form a triple helix and characterize the structural subunits of type IV collagen. All C-terminal ends of the alpha-chains form a globular domain that can be dissolved from the triple helix by treatment with bacterial collagenase.

The reactivity of Goodpasture specific anti-GBM autoantibodies is directed against the 29 kDa NC1 domain of the alpha-3 chain of type IV collagen of GBM. After the target antigen has been entirely characterized nowadays the triad of glomerulonephritis, lung hemorrhage and the anti-bodies against the α-3 chain of type IV collagen of GBM are the essential elements of diagnosis of Goodpasture syndrome. ELISA test systems provided with the corresponding pure antigen exhibit sensitivities and specificities of about 98 to 99%.

CONTENTS OF THE KIT

ORG 550   ▽ 96   Sufficient for 96 determinations

MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: **GBM**
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 20 U/ml, containing GBM antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 40 U/ml, containing GBM antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 80 U/ml, containing GBM antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 200 U/ml, containing GBM antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing GBM antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing GBM antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
STOP	15 ml	Stop solution; contains acid. Ready to use.
🔲i	1	Instruction for Use: ELISA Mini-DVD
🔲i	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 20 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute all patient samples **1:50** with sample buffer prior to use in the assay: combine **20 µl** of sample with 980 µl of

diluted sample buffer in a polystyrene tube. Mix well. Note: Calibrators / Controls need no dilution.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.

2. Dispense **100 µl** of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.

3. Dispense **100 µl** of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature

4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P2										
B	B	P3										
C	C											
D	D											
E	E											
F	C+											
G	C-											
H	P1											

P1, ... patient sample   A-E calibrators   C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.
Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is   0 - 200 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off   20 U/ml

Interpretation of results

Negative: < 20 U/ml

Positive: ≥ 20 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	175.7	175.7	100
.	1:200	89.0	87.9	101
.	1:400	43.2	43.9	98
.	1:800	21.8	22.0	99
.	1:1600	10.1	11.0	92
2	1:100	189.2	189.2	100
.	1:200	93.5	94.6	99
.	1:400	45.0	47.3	95
.	1:800	22.6	23.7	95
.	1:1600	11.0	11.8	93

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay				Inter-Assay		
Sample	Mean U/ml	CV %		Sample	Mean U/ml	CV %
1	15.6	5.3		1	14.2	6.4
2	85.8	5.4		2	90.1	5.4
3	143.4	5.7		3	154.4	5.8

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Goodpastures syndrome	20	20	100.0
Connective tissue disease	20	1	5.0
Autoimmune vasculitis	20	0	0.0
Normal human sera	100	0	0.0

Clinical Diagnosis

		Pos	Neg	
ORG 550	Pos	20	1	
	Neg	0	139	
		20	140	160

Sensitivity: 100.0 %
Specificity: 99.3 %
Overall agreement: 99.4 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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1 100 µl Standards, Kontrollen und verdünnte Patientenproben pipettieren
→ **30 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen

2 100 µl Enzymkonjugatlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen

3 100 µl Substratlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren

4 100 µl Stopplösung zugeben
→ Platte **5 Minuten** stehenlassen
→ Bei **450 nm** messen