Human GBM antibody ELISA Kit

Enzyme Immunoassay for the quantification of IgG antibodies to GBM in serum or plasma.

Catalog number: ARG80390

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INTRODUCTION

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 antiglomerular 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 -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 -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%.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified GBM has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard 0-4 (0, 20, 40, 80, 200 U/ml)	5 X 1.5 ml (Ready-to-use)	4°C.
Control 1 (Positive Control)	1 X 1.5 ml (Ready-to-use)	4°C
Control 2 (Negative Control)	1 X 1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled water before use.
- Patient sample: Dilute patient sample 1:50 with 1x Sample buffer before assay, mix well. (e.g. 20 µl serum + 980 µl 1x sample buffer Sample Buffer)

  Note: the controls / Standards are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100µl of standards, controls, samples and zero controls into wells.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100µl 1X HRP-antibody conjugate into each well. Incubate for 15 minutes at RT.

6. Wash as according to step 4.
7. Add 100µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
8. Add 100µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Negative: <20U/ml

Positive: >20U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of GBM Ab ranged from 20-200 U/ml. The mean MDD was 1 U/ml.

Interference

No interference has been observed with the following factors:

Haemolytic sera/plasma (up to 1000mg/dl)

Lipemic sera/plasma (up to 3g/dl triglycerides)

Bilirubin containing sera/plasma (up to 40mg/dl)

Antiboagulants (Citrate, EDTA, Heparin).

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.4% and inter-assay precision was 5.8%.