ImmuLisa™ Procedure at a Glance

Prepare Dilutions of Specimens

Pipette 100 µL of Specimens, Calibrators and Controls into Microwells

Incubate 30 Min. at Room Temperature

Wash Microplate 4x

Pipette 100 µL of Conjugate into Microwells

Incubate 30 Min. at Room Temperature

Wash Microplate 4x

Pipette 100 µL of Substrate Solution into Microwells

Incubate 30 Min. at Room Temperature

Pipette 100 µL of Stop Solution into Microwells

Read Absorbance at 405 NM

For technical assistance please contact:

IMMCO Diagnostics, Inc.
60 Pineview Drive
Buffalo, NY 14228-2120
Telephone: (716) 691-0091
Fax: (716) 691-0466
Toll Free USA/Canada: 1-800-537-TEST
E-Mail: info@immcodiagnostics.com

or your local product distributor

EU Authorized Representative/Autorisierter Repräsentant/Rappresentante Autorizzato/Representante Autorizado/Représentant Autorisé
EMERGO Group, Inc.
Molenstraat 15, 2513 BH, The Hague, The Netherlands
Tel (+31) 345 8570, Fax (+31) 346 7299
www.emergogroup.com

Anti-Glomerular Basement Membrane (GBM) Antibody ELISA

Product Insert

Catalog No. 1154
96 Determinations

Intended Use

An enzyme linked immunosorbent assay (ELISA) for the detection and semi-quantitation of anti-gglomerular basement membrane (GBM) antibodies in human serum. The presence of GBM antibodies can be used as an adjunct to clinical and other laboratory findings in the diagnosis of autoimmune renal disorders such as Goodpasture’s Syndrome.

Summary and Explanation

Rapidly progressive glomerulonephritis (RPGN) is a clinical syndrome developing over days or weeks characterized by crescentic glomerulonephritis on histopathology of the kidney. The prognosis is poor if not recognized early and if an appropriate treatment is not instituted. To optimize patient management, RPGN may be classified based on a) clinical assessment, b) direct immunofluorescence and electron microscopic studies of renal biopsy and c) serum antibody studies. Using the above criteria, RPGN may be classified into a) immune complex mediated disease characterized by the presence of anti-DNA antibodies or anti-streptococcal antibodies, b) anti-glomerular basement membrane (GBM) mediated glomerulonephritis and Goodpasture’s Syndrome and c) anti-neutrophil cytoplasmic antibody (ANCA) associated glomerulonephritis. In a study by Jayne et al1, of 889 RPGN suspected patients, 47 (5%) had anti-GBM, 246 (28%) had ANCA and 576 (65%) had neither antibodies. Two percent had both ANCA and anti-GBM antibodies. Anti-GBM antibodies can be detected by indirect immunofluorescence or by ELISA2-10. The antigen associated with anti-GBM antibodies is a non-collagenous domain of collagen IV.

Principles of Procedure

The ELISA test is performed in microwells coated with purified GBM antigen. Controls, calibrators and patient serum samples are incubated in the microwells allowing anti-GBM antibodies present in the serum to bind to the antigen. Unbound antibody and other serum proteins are removed by washing the microwells. Bound antibodies are incubated with an enzyme labeled anti-human IgG conjugate. Unbound conjugate is removed by washing the microwells. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of the substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in Enzyme Units per milliliter (EU/ml).
REAGENTS

Storage and Preparation
Store all reagents at 2°-8°C. Do not freeze.
Do not use if reagent is not clear or if a precipitate is present. All reagents must be
drawn to room temperature (20°-25°C) prior to use.
When stored at 2°-8°C, the reconstituted wash buffer is stable until the kit expiration
date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated
microwell strips are for one time use only.

Precautions
All human derived components used have been tested for HBsAg, HCV, HIV-1 and
2 and HTLV-I and found negative by FDA required tests. However, human blood
derivatives and patient specimens should be considered potentially infectious. Follow
good laboratory practices in storing, dispensing and disposing of these materials.
WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to
form highly explosive metal azides. Upon disposal of liquids, flush with large volumes
of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested,
report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure
valid results. Do not interchange kit components with those from other sources
other than the same catalog number from IMMCO DIAGNOSTICS. Follow good
laboratory practices to minimize microbial and cross contamination of reagents
when handling. Do not use beyond expiration date on the label.

Materials provided
ImmuLisa™ Anti-Glomerular-Basement Membrane (GBM) Antibodies Catalog No. 1154
Kit contains sufficient reagents to perform 96 determinations.

12 x 8 Ready to use Microplate with individual breakaway microwells coated
with GBM antigen.

1 x 1.5 ml *Ready to use Positive Control (red cap). Contains human serum
positive for anti-GBM antibodies. The expected concentration range in
EU/ml is printed on the label.

1 x 1.5 ml *Ready to use Negative Control (white cap). Contains human serum.

4 x 1.5 ml *Ready to use set of 4 Calibrators; Calibrator A (green cap), Calibrator
B (violet cap), Calibrator C (blue cap) and Calibrator D (yellow cap).
Human serum containing antibodies to GBM antigen. Concentrations
in EU/ml are printed on the label.

1 x 12 ml *Ready to use anti-human IgG Alk. Phos. Conjugate. Color coded
pink.

2 x 60 ml *Ready to use Serum Diluent. Color coded blue.

1 x 12 ml *Ready to use Enzyme Substrate. Contains pNPP. Protect from light.

1 x 12 ml Ready to use Stop Solution.

2 vials Powder Wash Buffer. Reconstitute to one liter each.

1 x extra Frame Holder
2 x Protocol Sheets

*CAUTION - Contains <0.1% NaN₃
Materials Required But Not Provided
• Deionized or distilled water
• Squeeze bottle to hold diluted wash buffer
• Pipettes capable of delivering 5 µl to 1000 µl
• Disposable pipette tips
• Clean test tubes 12 x 75 mm and test tube rack
• Timer
• Absorbent paper towels
• Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 630 nm.
• Automatic microplate washer capable of dispensing 200 µl

SPECIMEN COLLECTION AND HANDLING
Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2°- 8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

PROCEDURE

Procedural Notes
• Before starting with the assay read carefully the product insert.
• Let serum specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
• All dilutions of the patient samples should be prepared prior to starting with the assay.
• Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. An automated microplate washer is recommended.
• Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
• For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
• Addition of all samples and reagents should be performed at the same rate and in the same sequence.
• Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.
Test Method

Step 1 Let all reagents and specimens equilibrate at room temperature.

Step 2 Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.

Step 3 For a qualitative determination use only the Ready to Use Low Calibrator D (vial with yellow cap).

or For a semi-quantitative determination use the Ready to Use Calibrators A through D as depicted in the sample layout below.

### QUALITATIVE DETERMINATION

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### SEMI-QUANTITATIVE DETERMINATION

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Step 4 Prepare a 1:201 dilution of the patient samples by mixing 5 µl of the patient sera with 1.0 ml of Serum Diluent.

Step 5 Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder.

Step 6 Pipette 100 µl of Ready to use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.

Note: Include one well which contains 100 µl of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank.

Step 7 Incubate 30 minutes (± 5 min) at room temperature.

Step 8 Wash 4x with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer’s instructions.

Step 9 Pipette 100 µl of Conjugate into microwells.

Step 10 Incubate 30 minutes (± 5 min) at room temperature.

Step 11 Wash all microwells as in Step 8.

Step 12 Pipette 100 µl of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 13 Incubate 30 minutes (± 5 min) at room temperature.

Step 14 Pipette 100 µl of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance values within 1 hour from adding Stop Solution.

Step 15 Read absorbance of each microwell at 405 nm using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control
Calibrators, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3. The Calibrator A should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate, the mean of the two readings should be taken for determining EU/ml. While performing Qualitative determinations, the optical density of the Calibrator D must be greater than that of the negative control and lesser than the absorbance of the positive control. For semi-quantitative determinations, the positive control must give values in the range stated on the vial.

### RESULTS

Calculations
The concentrations of the patient samples can be determined by either of two methods:

1. **QUALITATIVE DETERMINATION**

   \[
   \text{Abs. of Test Sample} \times \frac{\text{EU/ml of Calibrator D}}{\text{Abs. of Calibrator D}} = \text{EU/ml Test Sample}
   \]

2. **SEMI-QUANTITATIVE DETERMINATION**

   Plot absorbance of Calibrator A through D against their respective concentration on a linear-linear graph paper. Plot the concentration in EU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Determine the concentrations of the patient samples from the curve against its corresponding absorbance value.

![Anti-GBM Immulisa™ Standard Curve](image)