

## GLOMERULAR BASEMENT MEMBRANE TEST SYSTEM

For In Vitro Diagnostic Use

AD GMK48 48 Tests  
AD GMK96 96 Tests

### Introduction:

Demonstration of Glomerular Basement Membrane antibodies (GBM), by immunofluorescent methods may enable serologic assessment and possible detection of kidney disease. The presence of a histologically defined circulating antibody to the glomerular basement membrane can aid in the patient diagnosis and possible diagnosis in Goodpasture's Syndrome. A linear fluorescence along the Glomerular Basement Membrane can be observed in most Goodpasture's patients.

### Principles:

The primary reaction involves circulating anti-glomerular basement membrane antibodies present in the patient's serum. The binding of the antibody to its homologous antigen site occurs during the first incubation period, while the serum covers the antigen surface. A second reaction follows after the unbound antibody is removed by a PBS rinse. The secondary reaction involves the binding of fluorescein labeled anti-human globulin, commonly referred to as conjugate. The unbound fluorescein conjugate then removed, by a PBS rinse, leaving only specifically bound sites to be observed through the microscope. Bright (3-4+) fluorescence of the glomerular basement membrane indicates a positive reaction. Care should be taken to locate vessel staining or staining outside the glomerulus, where smooth muscle activity could be accidentally reported as a positive reaction.

### Materials Provided:

Storage & Stability of Components:

1. FITC IgG H&L Conjugate No. AD CGEM2 (3.0 ml) with Evans Blue Counterstain is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
2. The antigen slides of monkey kidney sections must be stored at 2-8°C upon receipt. Check label for specific expiration date.
3. GBM positive control No. AD PCGB (1.0 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date.
4. Universal negative control No. AD NC (1.0 ml) should be stored at 2-8°C upon receipt. Check label for specific expiration date.
5. Buffer Pack No. AD PBS1 - Phosphate Buffered Saline is stable at room temperature storage. Check label for specific expiration date. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.
6. Mounting Medium No. AD TMM3 is stable when stored at 2-8°C. Check label for specific expiration date.

### Additional Materials Required but not Provided:

Test tubes and rack or microtiter system  
Disposable pipettes  
Staining Dish and Slide Forceps  
Moisture Chamber  
Volumetric Flask (500 ml)  
Distilled H<sub>2</sub>O  
Fluorescence Microscope  
Paper Towels - lint free

### Reagent Preparation:

1. Buffer Pack No. AD PBS1. Rehydrate buffer with 1 liter of sterile distilled water.

### Specimen Collection:

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

### Test Instruction:

**Screening:** dilute test serums 1:4 in PBS. **Titration:** set up doubling dilutions of serum starting at 1:4, 1:8, 1:16, 1:32, etc.

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30 µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 24°C).
4. Remove slide from moisture chamber and allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.
5. Wash in PBS for five minutes. Repeat using fresh PBS.
6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. **Do not allow tissue to dry.** Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.
7. Deliver 1 drop (20-30 µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope. Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

### Quality Control:

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.


3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

### Results:

A positive result is observed as a bright 3-4+ linear staining of the Glomerular basement membrane. This antibody is seen in many Goodpasture Syndrome patient, and may have significance in the patient profile, as well as aid the diagnosis and prognosis.

### Precautions:

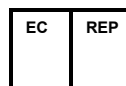
1. All human components have been tested by radioimmunoassay for (HB<sub>s</sub>A<sub>g</sub>) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB<sub>s</sub>A<sub>g</sub> or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
5. For In Vitro Diagnostic Use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.
8. Use separate pipette tips for each sample and reagent to avoid cross contamination.
9. Reagents should be inspected for evidence of bacterial or fungal contamination.
10. Do not reuse substrate slide.

<b>Component</b>	AD PBS1 PBS Powder Packets AD TMM3 Mounting Medium	<b>Precautionary Statement</b> <b>Prevention:</b> P264 Wash thoroughly after handling. P280 Wear protective gloves and clothing.
<b>Pictogram</b>		<b>Response:</b> P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if easy to do. Continue rinsing.
<b>Signal Word</b>	<b>WARNING</b>	P337+P313 If irritation persists, get medical advice/attention.
<b>Hazard Statement</b>	H319 Causes serious eye irritation	

### BIBLIOGRAPHY









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	Manufactured by Prodotto da Fabricado por Fabriqué par hergestellt von
<b>REF</b>	Catalog number Numero di catalogo Número de Catálogo Numéro de catalogue Katalognummer
<b>LOT</b>	Lot Lotto Lote Lot Charge
<b>EC REP</b>	EC Authorized Representative Rappresentante Autorizzato CE Representante Autorizado CE CE Représentant autorisé EG autorisierter Bevollmächtigter
	EC Declaration of Conformity Dichiarazione di Conformità CE Declaración de Conformidad CE CE Déclaration de Conformité EG Konformitätserklärung
	Number of tests Numero di test Número de determinaciones Nombre de tests Anzahl der Teste
	See instructions for use Vedere le istruzioni per l'uso Consultar la instrucciones de uso Voir instructions Gebrauchsanweisung beachten
	Expiration date Data di scadenza Caducidad Date d'Expiration Halbbarkeitsdatum
	Store at 2-8°C / 35-46°F Conservare a 2-8°C/35-46°F Almacenar a 2-8°C / 35-46°F Conserver à 2-8°C Bei 2-8°C / 35-46°F lagern
	Caution Attenzione Precaución Précautions Achtung
	Potential biological risk Potenziale rischio biologico Riesgo potencial biológico Biohazard Risque Biologique Potentiel Potentielle biologische Gefährdung
<b>RFU</b>	Ready for use Pronto all'uso Listo para su uso Prêt à l'emploi Gebrauchsfertig
<b>IVD</b>	For in vitro diagnostic use Per uso diagnostico <i>in vitro</i> Para uso solo in vitro Usage in vitro Für in-vitro diagnostische Verwendung
<b>RUO</b>	For research use only Solo per ricerca Para uso solo en investigación Pour recherche Nur für Forschungszwecke
<b>IUO</b>	For investigational use only Solo per uso investigativo Para uso solo en investigación Pour investigation Nur für Forschungszwecke
<b>IFA/DFA</b> <b>PBS</b>	Phosphate Buffered Saline Tampone salino fosfato Fosfato Salino Tamponado Tampon phosphate salin PBS
<b>SOR</b>	Sorbent Assorbent Sorbente Absorbant Sorbens

<b>SLIDE</b>	Tissue Substrate Slide Vetrini con substrate di tessuto Porto objetos de Substrato de Tejido Lame portant le substrat tissulaire Gewebesubstrat-Objekträger
<b>MM</b>	Mounting Medium Mezzo di montaggio Medio de Montaje Liquide de montage Eindeckmedium
<b>10x</b>	Concentration Concentrazione Concentración Concentration Konzentration
<b>ENS</b>	Enhancement solution Soluzione di rinforzo Solución de realce Solution d'amplification Verstärkungslösung
<b>WASHB</b>	Wash Buffer Tampone di lavaggio Tampón de lavado Tampon de lavage Waschpuffer
<b>MPS 12x8</b>	Microplate Strips Strip per Micropiastra Tiras de micro placa Microplaque Mikrotiterplattenstreifen
<b>CONJ</b>	Conjugate Coniugato Conjugado Conjugué Konjugat
<b>SUB</b>	Substrate Substrato Sustrato Substrat Substrat
<b>STOP</b>	Stop Solution Soluzione bloccante Solución de Parada Solution d'arrêt Stopplösung
<b>CAL X</b>	Calibrator(s) Calibratore (i) Calibrador (s) Calibrateur(s) Kalibrator(en)
<b>CONTROL -</b>	Negative Control Controllo Negativo Control Negativo Contrôle Négatif Negative Kontrolle
<b>CONTROL +</b>	Positive Control Controllo Positivo Control Positivo Contrôle Positif Positive Kontrolle
<b>CONJ CNS</b>	Counterstain Colorante di contrasto Contraste Contre colorant Gegenfärbung
<b>CS</b>	Coverslip Coprioggetto Cubre portaobjetos Lamelles couvre-objet Deckgläser
<b>CONJ +</b>	Positive Conjugate Coniugato Positivo Conjugado Positivo Conjugué Positif Positivekinjugat
<b>CONJ -</b>	Negative Conjugate Coniugato Negativo Conjugado Negativo Conjugué Négatif Negativekinjugat
<b>DIL</b>	Sample Diluent Diluente del campione Diluyente de muestra Tampon de dilution Probenverdünnungslösung