GBM ANTIGEN

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ATG02-02 GBM antigen 0.20 mg
ATG02-10 GBM antigen 1.0 mg

Description of the Product

Purified from bovine kidney. After coating onto ELISA plates the product will bind autoantibodies to GBM antigen.

Purity: The GBM antigen (NC1 domain of type IV collagen from glomerular basement membrane) is more than 90% pure, as assessed by SDS polyacrylamide gel electrophoresis.

Storage: Store at -65ºC or below (long term). Avoid repeated freezing and thawing. Mix thoroughly before use.

Clinical and Biochemical Data

Autoantibodies to glomerular basement membrane (GBM) are known to mediate the rapidly progressive glomerulonephritis (with or without pulmonary haemorrhage) typical of Goodpasture syndrome[1,2]. Assay of anti-GBM antibodies is valuable in three clinical conditions[3]: a) patients with acute renal failure where post- and pre-renal causes seem unlikely; b) patients with increasing serum creatinine levels together with haematuria and casts, and c) patients with pulmonary haemorrhage. Early detection of anti-GBM antibodies in such conditions followed by immediate immunotherapy may greatly improve the disease prognosis[4,5]. Patients with Goodpasture syndrome can exhibit clinical features typical of autoimmune vasculitic conditions. It has therefore been recommended that anti-GBM antibodies be assayed together with anti-neutrophil cytoplasmic antibodies (ANCA) in pulmonary renal syndromes[6,7].

Anti-GBM antibodies distribute along the GBM in a linear fashion as seen by direct immunofluorescence microscopy[8], indicative of reactivity with a specific GBM antigen that has been identified as the NC1 domain of α3 type IV collagen[9]. Collagen IV interweaves with laminins, nidogen and sulphated proteoglycans to assemble basement membranes[10]. In glomerular basement membrane three distinct α chains (α3.α4.α5) of type IV collagen assemble as a triple helical protomer with their respective C-terminal non-collagenous domains (NC1) forming a trimer at one extreme[11,12]. Two NC1 trimers unite to form a hexamer; these hexamers create collagenous networks by uniting four triple helical 7S domains at the N-terminal[12]. Super-coiling and disulphide bridges further stabilise the network. Two dominant epitopes have been identified on α3(IV) NC1, both of which are cryptic in nature and inaccessible for autoantibody binding unless dissociation of the NC1 hexamer occurs[13-15]. The epitopes appear to be conformation-dependent and are destroyed by reduction of disulphide bridges[3,16].

The NC1 domain of type IV collagen purified from bovine kidney glomeruli constitutes AroTec's GBM antigen. As such it is comprised of α3.α4.α5(IV) NC1 subunits and is depleted of α1(IV) NC1 against which antibodies have been reported to exist in patients with a paraneoplastic syndrome[17]. Bovine α3(IV) NC1 shares a high degree of amino acid sequence homology with its human counterpart[18], and its use in the detection of anti-GBM autoantibodies has been reported previously by several authors[7,19-21].

Methodology

The following is an ELISA procedure which can be used to detect anti-GBM autoantibodies in human serum using the ATG02- purified GBM antigen:

1. Dilute the purified antigen to 1.0-2.0 μg/ml in coating buffer (20 mM Tris-HCl, pH 8.0; 6.0 M guanidinium hydrochloride).
2. Coat ELISA plates with 100 μl of diluted antigen per well. Cover and incubate overnight at +4 ºC.
3. Empty the plates and remove excess liquid by tapping on a paper towel.
4. Block excess protein binding sites by adding 200 μl PBS (10 mM potassium phosphate, pH 7.4, 0.15 M NaCl) containing 1% BSA per well. Incubate at room temperature for three hours.
5. Empty plates and apply 100 μl of serum samples diluted 1:100 in PBS / 1% BSA / 1% casein / 0.1% Tween® 20. Incubate at room temperature for 1 hour.
6. Empty plates and add 200 μl PBS / 0.1% Tween® 20 per well. Incubate 5 minutes then empty plates. Repeat this step twice.
7. Apply 100 μl anti-human IgG-enzyme conjugate (horseradish peroxidase or alkaline phosphatase) diluted in PBS / 1% BSA / 1% casein / 0.1% Tween® 20 per well and incubate for 1 hour.
8. Repeat step 6.
9. Add enzyme substrate and stop the reaction when appropriate.
10. Read absorbance in an ELISA spectrophotometer.

References

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NOTE: **No patented technology** has been used by AROTEC during the preparation of this product.

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