

# GBM ANTIGEN

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ATG02-02	GBM antigen	0.20 mg
ATG02-05	GBM antigen	0.50 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.

**Concentration:** 0.1-1.0 mg protein/ml.

**Storage:** The product is stabilised with 0.1% Micr-O-protect™. Store at -20 °C or below (long term) or at +4 °C (short 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<sup>1,2</sup>. Assay of anti-GBM antibodies is valuable in three clinical conditions<sup>3</sup>: 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<sup>4,5</sup>. 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<sup>6,7</sup>.

Anti-GBM antibodies distribute along the GBM in a linear fashion as seen by direct immunofluorescence microscopy<sup>8</sup>, indicative of reactivity with a specific GBM antigen that has been identified as the NC1 domain of  $\alpha 3$  type IV collagen<sup>9</sup>. Collagen IV interweaves with laminins, nidogen and sulphated proteoglycans to assemble basement membranes<sup>10</sup>. In glomerular basement membrane three distinct  $\alpha$  chains ( $\alpha 3, \alpha 4, \alpha 5$ ) 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<sup>11,12</sup>. Two NC1 trimers unite to form a hexamer; these hexamers create collagenous networks by uniting four triple helical 7S domains at the N-terminal<sup>12</sup>. Super-coiling and disulphide bridges further stabilise the network. Two dominant epitopes have been identified on  $\alpha 3(\text{IV})$  NC1, both of which are cryptic in nature and inaccessible for autoantibody binding unless dissociation of the NC1 hexamer occurs<sup>13-15</sup>. The epitopes appear to be conformation-dependent and are destroyed by reduction of disulphide bridges<sup>3,16</sup>.

The NC1 domain of type IV collagen purified from bovine kidney glomeruli constitutes AroTec's GBM antigen. As such it is comprised of  $\alpha 3, \alpha 4, \alpha 5(\text{IV})$  NC1 subunits and is depleted of  $\alpha 1(\text{IV})$  NC1 against which antibodies have been reported to exist in patients with a paraneoplastic syndrome<sup>17</sup>. Bovine  $\alpha 3(\text{IV})$  NC1 shares a high degree of amino acid sequence homology with its human counterpart<sup>18</sup>, and its use in the detection of anti-GBM autoantibodies has been reported previously by several authors<sup>7,19-21</sup>.

## 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  $\mu\text{g/ml}$  in coating buffer (20 mM Tris-HCl, pH 8.0; 6.0 M guanidinium hydrochloride).
2. Coat ELISA plates with 100  $\mu\text{l}$  of diluted antigen per well. Cover and incubate overnight at +4 °C.
3. Empty the plates and remove excess liquid by tapping on a paper towel.
4. Block excess protein binding sites by adding 200  $\mu\text{l}$  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  $\mu\text{l}$  of serum samples diluted 1:100 in PBS / 1% BSA / 1% casein / 0.1% Tween<sup>®</sup> 20. Incubate at room temperature for 1 hour.
6. Empty plates and add 200  $\mu\text{l}$  PBS / 0.1% Tween<sup>®</sup> 20 per well. Incubate 5 minutes then empty plates. Repeat this step twice.
7. Apply 100  $\mu\text{l}$  anti-human IgG-enzyme conjugate (horseradish peroxidase or alkaline phosphatase) diluted in PBS / 1% BSA / 1% casein / 0.1% Tween<sup>®</sup> 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

1. Lerner, R.A. et al. (1967) J. Exp. Med. **126**, 989
2. Lockwood, C.M. et al. (1976) Lancet **1**: 711
3. Hellmark, T. et al. (1997) Nephrol. Dial. Transpl. **12**, 646
4. Kline-Bolton, W. (1966) Kidney Int. **50**, 1753
5. Peters, D.K. et al. (1982) Trans. Proc. **14**, 513
6. Bygren, P. et al. (1992) Eur. J. Clin. Inv. **22**, 783
7. Westman, K.W.A. et al. (1997) Nephrol. Dial. Transpl. **12**, 1863
8. Sheer, R.L. & Grossman, M.A. (1964) Ann. Intern. Med. **60**, 1009
9. Saus, J. et al. (1988) J. Biol. Chem. **263**, 13374
10. Yurchenko, P.D. et al. (2002) Meth. Cell Biol. **69**, 111
11. Borza, D.B. et al. (2002) J. Biol. Chem. **277**, 40075
12. Hudson, B.G. et al. (2003) New Engl. J. Med. **348**, 2543
13. Borza, D.B. et al. (2000) J. Biol. Chem. **275**, 6030
14. Gunnarson, A. et al. (2000) J. Biol. Chem. **275**, 30844
15. David, M. et al. (2001) J. Biol. Chem. **276**, 6370
16. Hellmark, T. et al. (1996) Clin. Exp. Immunol. **105**, 504
17. Kalluri, R. et al. (1996) Ann. Intern. Med. **124**, 651
18. Ryan, J.J. et al. (1998) Nephrol. Dial. Transpl. **13**, 602
19. Hellmark, T. et al. (1997) J. Am. Soc. Nephrol. **8**, 376
20. Gunwar, S. et al. (1991) J. Biol. Chem. **266**, 15318
21. Saxena, R. et al. (1989) J. Immunol. Meth. **118**, 73

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



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