

PROTEINASE 3 (cANCA) ANTIGEN

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ATP02-02	Proteinase 3 (cANCA) antigen	0.20 mg
ATP02-05	Proteinase 3 (cANCA) antigen	0.50 mg
ATP02-10	Proteinase 3 (cANCA) antigen	1.0 mg

Description of the Product

Purified from human neutrophils from blood tested and found to be negative for HBs-antigen, anti-HIV1, anti-HIV2, anti-HCV, Lues and GPT. After coating onto ELISA plates the product will bind autoantibodies to proteinase 3 (cANCA) antigen.

Purity: The proteinase 3 autoantigen is more than 95% pure, as assessed by SDS 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 neutrophil cytoplasmic antigens (ANCA) were first described in 1982 by Davies et al.¹ Autoantibodies staining the nuclei or the perinuclear zone of neutrophils by indirect immunofluorescence are referred to as pANCA whereas those giving a clear cytoplasmic fluorescence are referred to as cANCA². The antigen recognised by most cANCA sera has been identified as proteinase 3 (also known as myeloblastin, p29 or AGP7)³⁻⁵. Autoantibodies to proteinase 3 are found in the sera of patients with active Wegener's granulomatosis⁶ and less often in other types of systemic vasculitis including microscopic polyangiitis, idiopathic crescentic glomerulonephritis, Churg-Strauss syndrome and polyarteritis nodosa⁷.

Proteinase 3 is a serine proteinase with proteolytic activity towards a range of substrates⁸. It is physiologically inhibited by α_1 -antitrypsin⁹. The protein has been described as having antibiotic¹⁰ and growth-promoting¹¹ properties and causing empysema when administered by tracheal insufflation¹². Proteinase 3 cDNA has been cloned and sequenced¹⁰, revealing the protein to be a basic 25 kDa glycoprotein of 228 amino acids³. The protein reveals a triplet of bands in the range 29-32 kDa when subjected to denaturing SDS-electrophoresis, probably caused by differing degrees of glycosylation¹⁴. Although proteinase 3 shows significant homology to elastase and cathepsin G, cANCA sera do not cross react with these serine proteases¹⁵. Autoantibodies to proteinase 3 appear to recognise conformational epitopes¹⁶ and have been reported to interfere with inactivation of the enzyme by α_1 -antitrypsin¹⁷ and to also inhibit proteolytic activity itself¹⁷. The use of purified proteinase 3 for the detection of cANCA by solid-phase ELISA has been described by several authors^{2,18-25}.

Methodology

The following is an ELISA procedure which can be used to detect anti-proteinase 3 autoantibodies in human serum using the ATP02 purified antigen:

1. Dilute the purified antigen to 0.5-1.0 µg/ml in 0.05 M carbonate buffer pH 9.5.
2. Coat ELISA plates with 100 µl of diluted antigen per well. Cover and incubate overnight at room temperature.
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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