

# PROTEINASE 3 (cANCA) ANTIGEN

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ATP02-02	Proteinase 3 (cANCA) antigen	0.20 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.

**Storage:** Store at -65°C or below (long 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.<sup>1</sup> 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 cANCA<sup>2</sup>. The antigen recognised by most cANCA sera has been identified as proteinase 3 (also known as myeloperoxidase, p29 or AGP7)<sup>3-5</sup>. Autoantibodies to proteinase 3 are found in the sera of patients with active Wegener's granulomatosis<sup>6</sup> and less often in other types of systemic vasculitis including microscopic polyangiitis, idiopathic crescentic glomerulonephritis, Churg-Strauss syndrome and polyarteritis nodosa<sup>7</sup>.

Proteinase 3 is a serine protease with proteolytic activity towards a range of substrates<sup>8</sup>. It is physiologically inhibited by  $\alpha_1$ -antitrypsin<sup>9</sup>. The protein has been described as having antibiotic<sup>10</sup> and growth-promoting<sup>11</sup> properties and causing emphysema when administered by tracheal insufflation<sup>12</sup>. Proteinase 3 cDNA has been cloned and sequenced<sup>10</sup>, revealing the protein to be a basic 25 kDa glycoprotein of 228 amino acids<sup>13</sup>. 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 glycosylation<sup>14</sup>. Although proteinase 3 shows significant homology to elastase and cathepsin G, cANCA sera do not cross react with these serine proteases<sup>15</sup>. Autoantibodies to proteinase 3 appear to recognise conformational epitopes<sup>16</sup> and have been reported to interfere with inactivation of the enzyme by  $\alpha_1$ -antitrypsin<sup>17</sup> and to also inhibit proteolytic activity itself<sup>17</sup>. The use of purified proteinase 3 for the detection of cANCA by solid-phase ELISA has been described by several authors<sup>2,18-25</sup>.

## 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  $\mu\text{g/ml}$  in 0.05 M carbonate buffer pH 9.5.
2. Coat ELISA plates with 100  $\mu\text{l}$  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  $\mu\text{l}$  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

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

