MYELOPEROXIDASE (pANCA) ANTIGEN

AROTEC_MPO_Product_Info.pdf Version/Date: B/04.05.26

ATM01-02 Myeloperoxidase (pANCA) antigen 0.20 mg
ATM01-10 Myeloperoxidase (pANCA) 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 myeloperoxidase (pANCA) antigen.

Purity: The myeloperoxidase 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.[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 cANCA[2]. The antigen recognised by most pANCA sera has been identified as myeloperoxidase[3]. Autoantibodies to myeloperoxidase are found in the sera of patients with various types of systemic vasculitis[4-8] including idiopathic crescentic glomerulonephritis, Churg-Strauss syndrome, microscopic polyangiitis and polarteritis nodosa. Anti-myeloperoxidase antibodies have also been reported in some patients with Wegener's granulomatosis[9], and occasionally in patients with rheumatoid arthritis[10] or inflammatory bowel disease[11].

The heme-containing glycoprotein myeloperoxidase is a major constituent of neutrophil azurophilic granules[12] and plays a major role in the oxygen-dependent microbicidal system of these cells. This enzyme catalyses the oxidation of chloride by hydrogen peroxide to produce the highly reactive reagent hypochlorous acid[13]. When isolated from mature neutrophil granulocytes[14], myeloperoxidase consists of a tetramer composed of two 59 kDa and two 12 kDa subunits that are evident under reducing SDS-electrophoresis. Myeloperoxidase cDNA has been cloned and sequenced[15], revealing that the protein is synthesised as a 80 kDa precursor before it is processed into the 59 and 12 kDa subunits of 467 and 113 amino acids respectively. Autoantibodies to myeloperoxidase appear to recognise conformational epitopes[16]. The use of purified myeloperoxidase for the detection of anti-myeloperoxidase autoantibodies by solid-phase ELISA has been described by several authors[2,5-7,10].

Methodology

The following is an ELISA procedure which can be used to detect anti-myeloperoxidase autoantibodies in human serum using the ATM01 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

1. Davies, D.J. et al. (1982) Br. Med. J. **285**, 606
2. Wiik, A. et al. (1994) Man. Biol. Markers of Dis. (Kluwer Acad. Publ.) A9:1-14
3. Falk, R.J. & Jennette, J.C. (1988) N. Engl. J. Med. **316**, 1651
4. Falk, R.J. et al. (1990) Neth. J. Med. **36**, 121-125
5. Lee, S.S. et al. (1990) Clin. Exp. Immunol. **79**, 41-46
6. Cohen Tervaert, R. et al. (1990) Arthr. Rheum. **33**, 1264
7. Gueirard, P. et al. (1991) J. Autoimmun. **4**, 517
8. Ulmer, M. (1992) Clin. Nephrol. **37**, 161
9. Kallenberg, C.G.M. et al. (1994) Kidney Int. **46**, 1
10. Cambridge, G. et al. (1994) Ann. Rheum. Dis. **53**, 24
11. Snook, J.A. (1989) Clin. Exp. Immunol. **76**, 30
12. Klebanoff, S.J. (1968) J. Bacteriol. **95**, 2131
13. Harrison, J.E. & Schultz, J. (1976) J. Biol. Chem. **251**, 1371
14. Olsen, R.L. & Little, C. (1984) Biochem. J. **222**, 701
15. Falk, R.J. et al. (1992) Clin. Exp. Immunol. **89**, 274

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

AROTEC DIAGNOSTICS