BPI ANTIGEN

ATB01-02 BPI antigen 0.20 mg
ATB01-10 BPI antigen 1.0 mg

Description of the Product

Purified from human neutrophils. After coating onto ELISA plates the product will bind autoantibodies to BPI antigen.

Purity: The BPI antigen (55 kDa) is more than 90% pure, as assessed by SDS-polyacrylamide gel electrophoresis.

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

Clinical and Biochemical Data

The existence of autoantibodies to bactericidal/permeability-increasing protein (BPI) was first described when it was noticed that 11 of 51 sera that were cANCA positive in immunofluorescence recognised a 57 kDa antimicrobial cationic protein known as CAP57 in both ELISA and Western blot[1]. It is now known that CAP57 and BPI are identical[2]. More recently BPI purified from neutrophil granula was used to directly demonstrate the existence of anti-BPI autoantibodies in a range of vasculitic disorders by ELISA and Western blot[3]. BPI-ANCA have since been demonstrated to be present in a range of clinical conditions including ulcerative colitis[4,7,9,14,17,18,19], Crohn's disease[4,14,19], cystic fibrosis[4,5,10,15,16,20], rheumatoid arthritis[4,9], systemic lupus erythematosus[4,11], mixed connective tissue disease[4], Behcet's disease[6], bronchieactasis[12], primary sclerosing cholangitis[13,14,17] and chronic liver diseases[8,14].

BPI is a highly cationic protein[21] that is stored in the azurophil granula of neutrophils[22]. BPI is a potent antibacterial agent with selective toxicity towards Gram-negative bacteria[23]. It binds to the lipopolysaccharides present in the outer envelope of these bacteria to exert immediate bacteriostatic effects and later, bactericidal effects[24]. Analysis of the activity of proteolytic fragments[25] and of the crystal structure of BPI[26] reveal that the protein consists of two functionally distinct domains: a potent antibacterial and anti-endotoxin 20 kDa domain at the amino-terminal and a carboxyl terminal domain that is responsible for BPI's opsonic activity. Epitope studies using a panel of recombinant truncated BPI proteins indicate that anti-neutrophil cytoplasmic antibodies to BPI recognise the carboxyl terminal domain[27-29]. It has also been suggested elsewhere[29,30] that BPI-ANCA binding sites are likely to be conformational epitopes, as is the case with autoantibodies to proteinase 3 and myeloperoxidase. AroTec's BPI antigen is native protein purified from human neutrophils; the use of purified native BPI for the detection of BPI-ANCA by solid-phase ELISA has been described elsewhere[3,19,31].

Methodology

The following is an ELISA procedure which can be used to detect anti-BPI autoantibodies in human serum using the ATB01 purified BPI autoantigen:

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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