CENP-B ANTIGEN

AROTEC_CENP-B_Product_Info.pdf Version/Date: A/08.04.09

ATC02-02 CENP-B antigen 0.20 mg
ATC02-10 CENP-B antigen 1.0 mg

Description of the Product

Purified recombinant full-length human sequence protein. After coating onto ELISA plates the product will bind autoantibodies to CENP-B.

Purity: The CENP-B autoantigen (80 kDa) is more than 90% pure, as assessed by SDS-polyacrylamide gel electrophoresis.

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

Clinical and Biochemical Data

Autoantibodies to centromeric proteins in the sera of patients with scleroderma were first described in 1980[1]. Subsequent studies confirmed that anti-centromere antibodies (ACA) were most often found in patients with a limited form of systemic sclerosis previously referred to as CREST syndrome[2-5]. These autoantibodies are also often detected in sera from patients with Raynaud's phenomenon and occasionally in other rheumatic diseases such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis[6-10]. ACA have also been reported to occur with high prevalence in patients with primary biliary cirrhosis[11], in patients with malignancies[12-14] and occasionally in normal individuals[15,16].

Although at least 9 proteins are known to be associated with the centromere complex, CENP-B is normally considered to be the major centromere antigen, since autoantibodies to this protein were found to be present at high titres in all ACA sera while titres of antibodies directed at two other centromere antigens (CENP-A, CENP-C) were often much lower[17]. CENP-B is a dimer composed of two 80-kDa subunits[18] which binds specifically to the 17bp CENP-B box sequence[19] that is required for the formation of a functional centromere and is located within chromosomal α-satellite DNA. CENP-B may therefore function as a trans-acting factor that regulates the formation or centromere-specific chromatin on the α-satellite DNA repeat at the nuclesome assembly level[20]. Autoantibodies have been reported to bind to at least 3 different epitope regions of CENP B however the 60 amino acid C-terminal region appears to constitute the major autoimmune epitope[21].

Recombinant full-length human-sequence protein expressed and purified in Sf21 insect cells constitutes AROTEC's CENP-B antigen. The use of recombinant CENP-B antigen for the detection of anti-centromere antibodies has been described elsewhere[25-25].

Methodology

The following is an ELISA procedure which can be used to detect anti-centromere autoantibodies in human serum using the ATC02 purified CENP-B autoantigen:

1. Dilute the purified antigen to 0.1-0.5 μg/ml in 10 mM Tris-HCl, pH 7.4; 0.15 M NaCl
2. Coat ELISA plates with 100 μl of diluted antigen per well. Cover and incubate 24 hours at +4ºC.
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 containing 1% BSA per well. Cover and incubate at +4ºC overnight.
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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