Scl-70 ANTIGEN

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ATS01-02 Scl-70 antigen 0.20 mg
ATS01-10 Scl-70 antigen 1.0 mg

Description of the Product

Purified from bovine thymus. After coating onto ELISA plates the product will bind autoantibodies to Scl-70.

Purity: The Scl-70 autoantigen (75-82 kDa) is more than 90% pure, as assessed by SDS gel electrophoresis.

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

Clinical and Biochemical Data

Scleroderma (progressive systemic sclerosis) is a systemic autoimmune disease characterised by collagen deposition and connective tissue destruction of the skin, blood vessels and certain internal organs[1]. The prevalence of scleroderma has been estimated to be 1 per 5000[2]. Approximately 20-28% of scleroderma patients have autoantibodies to a nuclear protein referred to as Scl-70[3]. Anti-Scl-70 antibodies are found with a greater frequency in systemic sclerosis patients with proximal skin involvement than in systemic sclerosis patients with limited skin involvement[4].

In 1986 the Scl-70 antigen was identified by Shero et al.[5] to be the superhelical DNA-relaxing enzyme topoisomerase I. This report has since been confirmed by others[6,7]. The molecular weight of enzymatically-active topoisomerase I determined by SDS-electrophoresis has been reported to range from 67-100 kDa[8]. Recent data suggest that the 100 kDa form is the predominant form *in vivo*, and that the lower molecular weight forms are due to proteolytic degradation[9]. Both the native and lower molecular weight forms have been shown to effectively bind anti-Scl-70 autoantibodies[10].

The amino acid sequence of human Scl-70 antigen (DNA topoisomerase I) has recently been described after cloning and sequencing its cDNA[11]. The deduced molecular weight of the mature protein is 90,649 kDa; lower than the SDS-electrophoretic molecular weight. This may be due to post-translational processing or to anomalous electrophoretic behaviour of the protein. The protein was found to be very basic, with a calculated isoelectric point of 10.05, and to possess its catalytic activity within a 67.7 kDa C-terminal fragment. Although the sequence of bovine Scl-70 has not been determined, the utility of the bovine antigen for the detection of autoantibodies to Scl-70 in patient sera has been demonstrated by several authors[10, 12-14].

Methodology

The following is an ELISA procedure which can be used to detect anti-Scl-70 autoantibodies in human serum using the ATS01 purified antigen:

1. Dilute the purified antigen to 0.5-1.0 µg/ml in PBS (10 mM potassium phosphate, 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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NOTE: **No patented technology** has been used by AROTEC during the preparation of this product.

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