

Sm ANTIGEN

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ATS02-02 Sm antigen 0.20 mg

ATS02-10 Sm antigen 1.0 mg

Description of the Product

Purified from bovine thymus. After coating onto ELISA plates the product will bind autoantibodies to Sm antigen.

Purity: The Sm autoantigen is more than 90% 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 directed against the Sm autoantigen, named after a prototype serum, were first identified in 1966 using immunodiffusion¹. Anti-Sm antibodies are found in the sera of approximately 25% of all patients with systemic lupus erythematosus (SLE)² and their presence is considered to be a very specific marker of this disease³. Autoantibodies to Sm are also known to often occur in combination with autoantibodies to other antigens^{2,4}, including DNA, histone, RNP, SSA(Ro) and SSB(La).

The term "Sm antigen" is now known to be synonymous with at least nine different polypeptides⁵. These proteins are also known as the common or core proteins of snRNP particles⁶. snRNPs are a group of nuclear particles comprised of several polypeptides associated with a small nuclear RNA molecule⁷. The most abundant snRNPs are involved in pre-mRNA-splicing⁷. Since they bind to proteins common to different snRNP particles (B, D, E, F and G subunits), autoantibodies directed against Sm are able to precipitate a wide range of snRNAs^{8,9}. While the major Sm autoantigen is believed to be represented by the D polypeptide^{6,10-11}, the B and D proteins are known to share at least one epitope based on monoclonal antibodies with multiple specificities^{6,12}. Sequence comparison has shown that all the known Sm proteins share two evolutionarily conserved structural sequence motifs¹³⁻¹⁴, a possible explanation for their immunological cross-reactivity.

Sm D polypeptide subtypes¹⁵ represent the most abundant components of AroTec's Sm antigen. It has been shown elsewhere¹⁶ that the D polypeptide exhibits a much higher specificity for Sm autoantibodies than the B polypeptide does. Other Sm subunits (E,F,G) are detectable. Although the human Sm antigen sequences are known⁵, there is currently no data available for bovine antigens. However the very high degree of homology between human²⁰ and porcine²¹ Sm D2 sequences and the complete identity of human²² and mouse²³ Sm D1 would indicate that such antigens are highly conserved between mammalian species. The use of bovine antigen for the detection of anti-Sm antibodies has been described by several authors¹⁷⁻¹⁹.

Methodology

The following is an ELISA procedure which can be used to detect anti-Sm autoantibodies in human serum using the ATS02 purified Sm 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 +4°C.
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 +4°C 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.

