

# Sm ANTIGEN

AROTEC\_Sm\_Product\_Info.pdf Version/Date: B/04.05.20

ATS02-02	Sm antigen	0.20 mg
ATS02-05	Sm antigen	0.50 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.

**Concentration:** 0.1-1.0 mg protein/ml.

**Storage:** The product is stabilised with 20% glycerol and 0.1% Micr-O-protect™. Store at -20 °C or below (long term) or at +4°C (short 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 immunodiffusion<sup>1</sup>. Anti-Sm antibodies are found in the sera of approximately 25% of all patients with systemic lupus erythematosus (SLE)<sup>2</sup> and their presence is considered to be a very specific marker of this disease<sup>3</sup>. Autoantibodies to Sm are also known to often occur in combination with autoantibodies to other antigens<sup>2,4</sup>, including DNA, histone, RNP, SSA(Ro) and SSB(La).

The term "Sm antigen" is now known to be synonymous with at least nine different polypeptides<sup>5</sup>. These proteins are also known as the common or core proteins of snRNP particles<sup>6</sup>. snRNPs are a group of nuclear particles comprised of several polypeptides associated with a small nuclear RNA molecule<sup>7</sup>. The most abundant snRNPs are involved in pre-mRNA-splicing<sup>7</sup>. 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<sup>8,9</sup>. While the major Sm autoantigen is believed to be represented by the D polypeptide<sup>6,10-11</sup>, the B and D proteins are known to share at least one epitope based on monoclonal antibodies with multiple specificities<sup>6,12</sup>. Sequence comparison has shown that all the known Sm proteins share two evolutionarily conserved structural sequence motifs<sup>13-14</sup>, a possible explanation for their immunological cross-reactivity.

Sm D polypeptide subtypes<sup>15</sup> represent the most abundant components of AroTec's Sm antigen. It has been shown elsewhere<sup>16</sup> 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 known<sup>5</sup>, there is currently no data available for bovine antigens. However the very high degree of homology between human<sup>20</sup> and porcine<sup>21</sup> Sm D2 sequences and the complete identity of human<sup>22</sup> and mouse<sup>23</sup> 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 authors<sup>17-19</sup>.

## 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.

  
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