

La (SSB) ANTIGEN

AROTEC_La-SSB_Product_Info.pdf Version/Date: B/04.05.20

ATL01-02	La (SSB) antigen	0.20 mg
ATL01-05	La (SSB) antigen	0.50 mg
ATL01-10	La (SSB) antigen	1.0 mg

Description of the Product

Purified from bovine thymus. After coating onto ELISA plates the product will bind autoantibodies to La (SSB).

Purity: The La autoantigen (45-50 kDa) is more than 90% pure, as assessed by SDS polyacrylamide 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

Sjögren's syndrome (SS) is a common systemic autoimmune inflammatory disorder characterised by lymphocyte-mediated destruction of exocrine glands leading to diminished or absent glandular secretion^{1,4}. SS may present as a primary disease or in association with other systemic autoimmune diseases (referred to as secondary SS). Autoantibodies to the La (SSB) antigen can be detected in the sera of up to 87% of patients with primary or secondary SS^{5,6}. The presence of anti-La (SSB) autoantibodies usually coincides with the presence of anti-Ro (SSA) autoantibodies⁷, however the fact that anti-Ro autoantibodies are far more common in other rheumatological conditions such as systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) suggests that anti-La is more specific for primary and secondary SS than anti-Ro^{8,9}. Anti-La autoantibodies have also been reported to be present in other clinical conditions, most notably in the sera of mothers of infants with neonatal lupus syndrome¹⁰, but also in 10 to 15% of SLE patients^{11,12}.

La (SSB) antigen binds to the oligo(U) 3' termini of nascent RNA polymerase III transcripts and facilitates transcriptional termination and reinitiation by this enzyme^{13,17}. It has also been reported to function as an ATP-dependent helicase able to melt RNA-DNA hybrids¹⁸. La (SSB) may be involved in other processes as well such as maturation and/or nuclear export of RNA polymerase III products and some aspects of translation^{19,20}. La (SSB) is a highly phosphorylated protein which migrates at about 50 kDa in SDS-polyacrylamide gel electrophoresis²¹. Phosphorylated residues are present at the carboxy-terminal part of the protein²². At least 8 isoelectric forms (pI range 6 to 7) have been identified²³.

The amino acid sequences of both human and bovine La (SSB) antigen have been determined by cDNA cloning and sequencing^{19,28}. Comparison of the two sequences shows 22 largely conservative amino acid substitutions with a total of 95% identity. Three regions of the La molecule (amino acids 1-107, 111-242 and 346-408) are thought to contain the major epitopes reactive with human anti-La sera^{19,24}. The broad cross-reactivity of patient sera with La (SSB) from diverse mammalian species indicates the presence of conserved epitopes²⁵. The use of bovine La (SSB) antigen for the detection of human anti-La (SSB) antibodies has been described by several authors²⁵⁻²⁷.

Methodology

The following is an ELISA procedure which can be used to detect anti-La (SSB) autoantibodies in human serum using the ATL01 purified autoantigen:

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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