RNP/Sm ANTIGEN

ATR01-02 RNP/Sm antigen 0.20 mg
ATR01-10 RNP/Sm antigen 1.0 mg

Description of the Product

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

Purity: The RNP/Sm autoantigen 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

Autoantibodies directed against the snRNP (small nuclear ribonucleoprotein) autoantigens referred to as RNP and Sm were originally detected in the sera of systemic lupus erythematosus (SLE) patients[1,2]. Anti-RNP antibodies were subsequently found in the sera of mixed connective tissue disease (MCTD) patients[3], and it is now known that when these antibodies occur at high titre and in the absence of Sm, they are a good marker for MCTD[3,4]. Such autoantibodies are also known to occur in the sera of patients with a range of other rheumatic diseases including progressive systemic sclerosis, rheumatoid arthritis, discoid lupus erythematosus, Sjögren's syndrome and various overlapping conditions[5-10].

The snRNPs are a group of nuclear particles comprised of several polypeptides associated with a small nuclear RNA molecule[11]. The most abundant snRNPs are involved in pre-mRNA-splicing[11]. At least 13 different snRNAs have been identified in mammalian cells[11]. Whereas autoantibodies directed against Sm are able to precipitate a wide range of snRNAs, RNP autoantibodies are only able to precipitate one particular type, referred to as U1snRNA. Anti-RNP antibodies react with the U1 snRNP-specific polypeptides[12] (68K, A and C antigens) whereas anti-Sm antibodies react with polypeptides associated with U1, U2, U5 and U4/U6 snRNAs (B,B', D, E, F and G antigens)[12,13]. While the 68K polypeptide constitutes the major MCTD RNP autoantigen[14,15], the major Sm autoantigen is represented by the D polypeptide[14-16].

The RNP 68K (present as a 33/35K doublet), RNP A and Sm D polypeptides represent the most abundant components of AroTec's RNP/Sm antigen. Other snRNP subunits (RNP-C) are also detectable. The antigen typically exhibits a 260nm/280nm absorbance ratio of >1.5, suggesting that a significant snRNA component is present. Although the human RNP and Sm antigen sequences are known[17,18], there is currently no data available for bovine antigens. However the very high degree of homology between human[19] and porcine[20] Sm D_2 sequences and the complete identity between human and mouse RNP 68K and Sm D1 sequences[21-24] would indicate that such antigens are highly conserved between mammalian species.

Methodology

The following is an ELISA procedure which can be used to detect anti-RNP or anti-Sm autoantibodies in human serum using the ATR01 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.