

RNP 68K ANTIGEN (Sm-FREE)

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ATR04-02	RNP 68K antigen	0.20 mg
ATR04-05	RNP 68K antigen	0.50 mg
ATR04-10	RNP 68K antigen	1.0 mg

Description of the Product

Purified from bovine thymus. After coating onto ELISA plates the product will bind autoantibodies to RNP 68K antigen.

Purity: The RNP 68K 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 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 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) patients³, 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 conditions⁵⁻¹⁰.

The snRNPs are a group of nuclear particles comprised of several polypeptides associated with a small nuclear RNA molecule¹¹. The most abundant snRNPs are involved in pre-mRNA-splicing¹¹. At least 13 different snRNAs have been identified in mammalian cells¹¹. 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 polypeptides¹² (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}.

Current ELISA methods for the detection of autoantibodies to RNP often require the simultaneous determination of reactivity to both RNP/Sm and Sm antigens. The reactivity with Sm is then subtracted from that with RNP/Sm, the difference being considered to be due to RNP-specific autoantibodies. The availability of RNP antigen in the absence of Sm would avoid the necessity for a dual assay to detect anti-RNP antibodies and assays have been reported using isolated U1-RNP specific subunits^{14,15}. Since the 68K U1-RNP polypeptide constitutes the major MCTD RNP autoantigen^{16,17}, efforts to produce a recombinant RNP antigen have focussed on this subunit^{18,20}, and not the U1-RNP specific subunits A and C which are known to share sequence homology and cross-reactivity with the Sm antigen B/B'²¹.

Native RNP 68K subunit (present as a 33/35K doublet) is the primary component of AroTec's RNP 68K antigen (Sm-free). Although the human RNP 68K sequence is known²², there is currently no data available for the bovine antigen. However the complete identity between human²² and mouse²³ sequences 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 68K autoantibodies in human serum using the ATR04 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 +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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