RIBOSOMAL P ANTIGEN

AROTEC_RiboP_Product_Info.pdf Version/Date: B/04.05.25

ATR03-02 Ribosomal P antigen 0.20 mg
ATR03-10 Ribosomal P antigen 1.0 mg

Description of the Product

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

Purity: The ribosomal P antigen subunits P0, P1 and P2 (38, 19 and 17 kDa respectively) are 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 ºC or below (long term) or at +4ºC (short term). Avoid repeated freezing and thawing. Mix thoroughly before use.

Clinical and Biochemical Data

The existence of autoantibodies to ribosomal components has been known for some time[1,2]. In 1985, two groups independently identified the ribosomal P proteins as the major protein antigens recognised by ribosomal antibodies[3,4]. Anti-ribosomal P antibodies are considered to be very specific for systemic lupus erythematosus (SLE)[5,6], and their presence frequently correlates with disease activity, in particular psychotic depression[6], hepatitis[7-9], and nephritis[10,11]. Although anti-ribosomal P antibodies have been reported to occur in patients with systemic sclerosis[12], this would appear to be rare and normally indicates an overlap with SLE[13].

The P proteins are three of approximately 80 proteins that make up the largest cytoplasmic ribonucleoprotein, the ribosome. Since ribosomes are assembled in the nucleoli, high titre anti-ribosomal P sera will show nucleolar as well as cytoplasmic immunofluorescent staining[14]. The exact functions of the individual P proteins are not fully understood however studies suggest that they collectively comprise part of a functional GTPase domain necessary for the binding of factor-GTP complexes[15,16]. This interaction results in catalysis of the appropriate step of the protein synthesis cycle (initiation, elongation or release). GTP is hydrolysed and the factor is released. The ribosomal P proteins are distinctive through their overall net negative charge, high content of alanine and predicted secondary structure (approx. 70% helical)[14]. The C-terminal 17 amino acids of all three P proteins are virtually identical and are highly conserved between species. Although the major autoantibody epitope on all three proteins is believed to be located within the C-terminal 22 amino acids[14,17], there is recent evidence that other individual P protein-specific epitopes occur[18].

The ribosomal P0, P1 and P2 proteins are all present in AroTec's ribosomal P antigen. The antigen typically exhibits a 260/280 nm absorbance ratio of >1.5, suggesting that a significant rRNA component is present. The sequences of the bovine P0 and P2 proteins have been determined[19], and found to be very homologous (>99%) to their human equivalents[20]. In particular the C-terminal 22 amino acids of both species were found to be identical.

Methodology

The following is an ELISA procedure which can be used to detect anti-ribosomal P autoantibodies in human serum using the ATR03 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 +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.

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