

Ro (SSA) ANTIGEN

AROTEC_Ro-SSA_Product_Info.pdf Version/Date: B/04.05.20

ATR02-02	Ro (SSA) antigen	0.20 mg
ATR02-05	Ro (SSA) antigen	0.50 mg
ATR02-10	Ro (SSA) antigen	1.0 mg

Description of the Product

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

Purity: The Ro autoantigen (60 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

Autoantibodies to the Ro(SSA) antigen are one of the most frequent serological markers of autoimmunity in rheumatic diseases^{1,2}. They are present in the serum of 50-80% of patients with Sjögren's syndrome (SS), 30-40% of patients with systemic lupus erythematosus (SLE), and 3-5% of patients with rheumatoid arthritis (RA)^{3,5}. Ro(SSA) autoantibodies have also been reported to be present in Sjögren's syndrome-lupus overlap disease, subacute cutaneous lupus erythematosus, lupus with complement component deficiencies, neonatal lupus syndrome, multiple myeloma, polymyositis, progressive systemic sclerosis and primary biliary cirrhosis⁶. The incidence of anti-Ro(SSA) antibodies in the population of normal women of reproductive age is in the order of 1%^{7,8}. The presence of anti-La(SSB) autoantibodies usually coincides with the presence of anti-Ro(SSA)^{9,10}.

Ro(SSA) antibodies target protein antigens associated with small RNA molecules known as hY-RNAs^{11,12}. These protein-RNA complexes are referred to as Ro-ribonucleoproteins (Ro-RNPs) and their biological function has yet to be elucidated¹³. It is generally acknowledged that a 60kDa protein (Ro60) intimately associated with Ro-RNP constitutes the major Ro(SSA) antigen¹². The binding of autoantibodies to Ro60 is heterogeneous: some sera recognise both the native and denatured antigen while others recognise the native antigen conformation only¹⁴⁻¹⁶. The association of a second putative Ro(SSA) antigen of 52kDa (Ro52) with Ro-RNP remains unclear. Although it has been proposed that Ro52 may be indirectly associated with Ro-RNP through Ro60¹⁷, it has been conversely demonstrated that Ro52 does not copurify with Ro-RNPs during chromatographic separation¹⁸. Furthermore true monospecific anti-Ro52 sera are known to be rare, they do not display a typical Ro(SSA) immunofluorescence pattern and are negative in immunodiffusion^{18,19}.

The Ro60 polypeptide represents the primary component of AroTec's Ro(SSA) antigen. The antigen typically exhibits a 260nm/280nm absorbance ratio of >1.3, suggesting that a significant hY-RNA component is present. Although the human Ro60(SSA) antigen amino acid sequence is known^{20,21}, there is currently no data available for the bovine antigen. However the very high degree of homology between human and mouse²² sequences indicates that Ro(SSA) antigen is highly conserved between mammalian species. The use of bovine antigen for the detection of anti-Ro(SSA) antibodies has been described elsewhere²³⁻²⁵.

Methodology

The following is an ELISA procedure which can be used to detect anti-Ro(SSA) autoantibodies in human serum using the ATR02 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

1. Tan, E.M. (1989) *Adv. Immunol.* **44**, 93
2. McCauliffe, D.P. & Sontheimer, R.D. (1993) *J. Inv. Dermatol.* **100**, 73S
3. Harley, J.B. et al. (1986) *Arthritis Rheum.* **29**, 196
4. Reichlin, M. (1986) *J. Clin. Immunol.* **6**, 339
5. Boire, G. et al. (1993) *J. Rheumatol.* **20**, 1654
6. Harley, J.B. et al. (1992) *Rheum. Dis. Clin. N. America* **18**, 337
7. Calmes, M. & Bartholomew, B.A. (1985) *J. Clin. Pathol.* **38**, 73
8. Taylor, P.V. et al. (1988) *Br. J. Rheumatol.* **27**, 126
9. Craft, J.E. & Hardin, J.A. (1987) *J. Rheumatol.* **14** S13, 106
10. St. Clair, J.P. et al. (1992) *Rheum. Dis. Clin. N. America* **18**, 359
11. Hendrick, J.P. et al. (1981) *Mol. Cell Biol.* **1**, 1138
12. Weilin, S.L. et al. (1984) **81**, 1996
13. Chan, E.K.L. & Buyon, J.P. (1994) *Man. Biol. Markers Dis.* (Kluwer Acad. Publ.) **B4**, 1/1-18
14. Boire, G. et al. (1991) *Arthritis Rheum.* **34**, 722
15. Itoh, Y. & Reichlin, M. (1992) *Autoimmunity* **14**, 57
16. Tsuzaka, K. et al. (1994) *Arthritis Rheum.* **37**, 88
17. Slobbe, R.L. et al. (1992) *J. Mol. Biol.* **227**, 361
18. Boire, G. et al. (1995) *Clin. Exp. Immunol.* **100**, 489
19. Meheus, L. et al. (1998) 4th Dresden Symp. Autoantibodies (Abstract)
20. Deutscher, S.L. et al. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9479
21. Ben-Chetrit, E. et al. (1989) *J. Clin. Invest.* **83**, 1284
22. Wang, D. et al. (1996) *Mol. Biol. Rep.* **23**, 205
23. Gaither, K.K. & Harley, J.B. (1985) *Prot. Biol. Fluids Proc. Coll.* **33**, 413
24. Yamagata, H. et al. (1984) *J. Clin. Invest.* **74**, 625
25. Yoshida, S. & Reichlin, M. (1990) *J. Immunol. Methods* **131**, 113

Micr-O-protect is from Roche Diagnostics GmbH (Mannheim, Germany).

Tween® 20 is a registered trademark of ICI Americas Inc. NOTE: **No patented technology** has been used by AroTec during the preparation of this product.



AROTEC DIAGNOSTICS LIMITED