Ro52 ANTGEN

ATR05-02  Ro52 antigen  0.20 mg
ATR05-10  Ro52 antigen  1.0 mg

Description of the Product
Recombinant full-length human sequence protein. After coating onto ELISA plates the product will bind autoantibodies to Ro52.

Purity: The Ro52 autoantigen (52 kDa) is more than 90% pure, as assessed by SDS-polyacrylamide gel electrophoresis.

Storage: Store at -65°C or below (long 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 autoimmune markers in rheumatic diseases. Other functional studies have suggested a role for a putative leucine zipper domain of Ro52 in protein dimer formation and inhibition of transcription activity, and involvement of Ro52 in the ubiquitin pathway. Although autoantibodies are known to bind to multiple epitopes on the Ro52 protein, the leucine zipper domain and an area on the N-terminal side of it have been shown to constitute a prominent linear epitope. Antibodies to the leucine zipper domain of Ro52 have been reported to bind to Ro60 in a conformationally-dependent fashion, suggesting there might be some degree of epitope spreading between Ro60 and Ro52.

Recombinant full-length human-sequence protein constitutes Arotec’s Ro52 antigen. The antigen has been expressed and purified in a denatured form – denatured Ro52 is known to react with Ro52-specific autoantibodies. The antigen has been expressed and purified in a denatured form – denatured Ro52 is known to react with Ro52-specific autoantibodies. The antigen was then reconstituted in a refolded form, which allows for the detection of antibodies to Ro52.

Methodology
The following is an ELISA procedure which can be used to detect anti-Ro52 autoantibodies in human serum using the ATR05 purified Ro52 autoantigen:

1. Dilute the purified antigen to 0.2-0.5 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.
9. Add enzyme substrate and stop the reaction when appropriate.
10. Read absorbance in an ELISA spectrophotometer.

References
22. Itoh, Y. et al. (1992) Autoimmunity 14, 89