Ro52 ANTIGEN

Product Datasheet

Description of the Product:
Recombinant full-length human sequence Ro52 protein with hexa-histidine tag. 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 & Handling:
Store at -65°C or below (long term). Avoid repeated freezing and thawing. Ro52 buffer contains SDS, this must be completely solubilized before use:

- Thaw at room temperature for at least an hour, OR
- Warm the vial above 19°C, OR
- Place the vial in a 37°C water bath for 5 minutes.

Gentle but thorough mixing is required before use.

Clinical and Biochemical Data:
Autoantibodies to the Ro(SSA) antigen are one of the most frequent serological autoimmune markers in rheumatic diseases[1,2] (refer also to AROTEC product ATR02 Ro(SSA) antigen). Anti-Ro-positive sera may contain two different types of autoantibody; those directed to a 60 kDa and those directed to a 52 kDa polypeptide component (referred to as Ro60 and Ro52 respectively)[3]. While the vast majority of Ro(SSA) positive sera react with both of these components, anti-Ro60 antibody has been reported to occur without anti-Ro52 antibody only in SLE sera[4,5] whereas anti-Ro52 antibody has been reported to occur in the absence of anti-Ro60 antibody in idiopathic inflammatory myopathy[6], dermatomyositis[7,8] and scleroderma[7,8]. Antibodies to Ro52 have been observed to occur not only monospecifically or with Ro(SSA)/La(SSB), but also concurrently with antibodies to Jo-1[6] and to other aminoacyl transferases[7], with anti-SRP[7], anti-PMScl[7], anti-CENP-B[9], anti-RNP[9] and antihistones[9]. The differing serological and clinical associations of anti-Ro52 and anti-Ro60 suggest that anti-Ro52 should be considered as an additional connective tissue disease serum marker[9], independent of anti-Ro60(SSA).

Ro52 is a member of the tripartite motif (TRIM) family of proteins. The TRIM motif includes three zinc-binding domains, a RING finger, a B-box type 1 and a B-box type 2, and a centre coiled-coil region (leucine zipper). TRIM proteins are believed to identify specific cell compartments through a process of homo-multimerisation[10,11]. Other functional studies have suggested a role for a putative leucine zipper domain of Ro52 in protein dimer formation and inhibition of transcription activity[12], and involvement of Ro52 in the ubiquitin pathway[13,14]. Although autoantibodies are known to bind to multiple epitopes on the Ro52 protein[15-17], the leucine zipper domain and an area on the N-terminal side of it have been shown to constitute a prominent linear epitope[18-21]. Antibodies to the leucine zipper domain of Ro52 have been reported to bind to Ro60 in a conformationally-dependent fashion[22,23] suggesting there might be some degree of epitope spreading between Ro60 and Ro52.

AROTEC's Ro52 antigen has been expressed and purified in a denatured form – denatured Ro52 is known to react well with Ro52-specific autoantibodies[22,24]. The use of recombinant antigen for the detection of anti-Ro52 antibodies has been described elsewhere[25,26].

Ordering information:
ATR05-02 Ro52 antigen 0.20 mg
ATR05-10 Ro52 antigen 1.0 mg

Custom pack sizes available on request

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.
8. Repeat step 6.
9. Add enzyme substrate and stop the reaction when appropriate.
10. Read absorbance in an ELISA spectrophotometer.

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