Jo-1 ANTIGEN

ATJ01-02 Jo-1 antigen 0.20 mg
ATJ01-10 Jo-1 antigen 1.0 mg

Description of the Product

Purified from bovine thymus. After coating onto ELISA plates the product will bind autoantibodies to Jo-1.

Purity: The Jo-1 autoantigen (55 kDa) is more than 90% pure, as assessed by SDS gel electrophoresis.

Storage: Store at -65ºC or below (long term). Avoid repeated freezing and thawing. Mix thoroughly before use.

Clinical and Biochemical Data

Polymyositis (PM) and dermatomyositis (DM) are idiopathic inflammatory myopathies characterised by proximal muscle weakness, elevated muscle enzyme activities and electromyographic and histological features[1,2]. The existence of autoantibodies to nuclear and cytoplasmic antigens, in particular to the aminoacyl-tRNA synthetase group of enzymes, in the sera of up to 89% of patients[3] indicates that these diseases have an autoimmune origin. The most common autoantibody in PM and DM is anti Jo-1, occurring in 15-20% of all myositis patients and about 30% of adult PM patients[4,5].

In 1983 the Jo-1 antigen was reported by Mathews and Bernstein[6] to be histidyl-tRNA synthetase (HRS), an enzyme which catalyses the coupling of histidine to its specific tRNA before transport to the ribosome followed by incorporation into a polypeptide chain during protein synthesis[7]. Patient autoantibodies are capable of inhibiting histidyl-tRNA synthetase activity and can immunoprecipitate labelled enzyme as well as its specific $tRNA^{his}$[6,8]. HRS has a molecular weight of 55 kDa in SDS-polyacrylamide electrophoresis, and is believed to exist as a dimer in its native state[9].

The amino acid sequence of human Jo-1 antigen has been determined by cloning and sequencing its cDNA[10]. It contains three structural motifs typical of class IIa aminoacyl transferases and two signature regions common to histidyl-tRNA synthetases. The human enzyme amino acid sequence shows significant homology to those of the enzymes from yeast (47.5%) and E. coli. It is predicted to have a coiled-coil α-helical structure that is characteristic of many autoantigens and which contains the major autoantigenic epitope[11]. Although the primary structure of bovine HRS has not yet been reported, the fact that mouse HRS[12] shares more than 98% sequence homology with the human enzyme suggests that there exists a high degree of homology between mammalian HRS sequences and that the bovine enzyme sequence is likely to be very similar to that of human HRS. The use of bovine antigen for the detection of anti-Jo-1 autoantibodies has been described elsewhere[13,14].

Methodology

The following is an ELISA procedure which can be used to detect anti-Jo-1 autoantibodies in human serum using the ATJ01 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 +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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