MITOCHONDRIAL ANTIGEN

AROTEC_MitoAg_Product_Info.pdf Version/Date: B/04.05.26

ATM02-02 Mitochondrial antigen 0.20 mg
ATM02-10 Mitochondrial antigen 1.0 mg

Description of the Product

Purified from bovine heart muscle. After coating onto ELISA plates the product will bind autoantibodies to mitochondrial antigen.

Purity: The mitochondrial antigen (5 subunits: PDH E2 (74 kDa), PDH E3 (Protein X, 56 kDa), PDH E3 (lipoamide dehydrogenase, 55 kDa), PDH E1α (41 kDa) and PDH E1β (36 kDa)) is more than 90% pure, as assessed by SDS polyacrylamide gel electrophoresis.

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

Clinical and Biochemical Data

Antimitochondrial antibodies (AMA) were first detected in sera from patients with primary biliary cirrhosis (PBC) using immunofluorescent staining on cryostat sections of a range of tissues[1]. PBC is characterised by a T-cell mediated inflammation of the medium-sized bile ducts and typically affects women over the age of forty[2]. The mitochondrial antigens of PBC have been identified as components of a functionally related enzyme family (the 2-oxo-acid dehydrogenase complexes) of which the pyruvate dehydrogenase (PDH) complex is the most prominent autoantigenic component[3-6]. Although autoantibodies to PDH are most frequently detected in the sera of patients with PBC, they have also been reported to occur in a minority of patients with rheumatic autoimmune diseases[7] (Sjögren's syndrome, scleroderma, SLE and rheumatoid arthritis) as well as in patients with leprosy[8], monoclonal gammopathies[9], tuberculosis[10] or anti-glomerular basement membrane disease[11].

2-Oxo-acid dehydrogenase complexes catalyse key reactions in intermediary metabolism[12,13]. The PDH complex oxidatively decarboxylates pyruvate to acetyl CoA; patient autoantibodies have been reported to inhibit enzyme function[14,15]. Each enzyme complex consists of multiple copies of three enzymes E1, E2 and E3. E2 forms a central symmetrical core to which multiple copies of E1, E3 and protein X are attached[16] yielding a total complex molecular weight of several thousand kDa. As is evident from primary sequence data of the human[16], pig[17] and rat[3,18] 70 kDa M2 antigen (PDH E2), that the PDH autoantigen is well conserved between species. PBC patient sera have been shown to react with PDH complexes from E. coli to human[19-22].

The PDH complex from bovine heart muscle constitutes AroTec's mitochondrial antigen. Four of the five subunits present (E2 acetyltransferase, E3 Protein X, E1α decarboxylase and E1β decarboxylase) are known to react with AMA[23]. The use of native PDH complex for the detection of AMA by ELISA has been described by several authors[11,24-26].

Methodology

The following is an ELISA procedure which can be used to detect antimitochondrial autoantibodies in human serum using the ATM02 purified mitochondrial antigen:

1. Dilute the purified antigen to 0.2-0.5 µg/ml in coating buffer (20 mM Tris-HCl, pH 8.0; 0.40 M NaCl; 0.05% deoxycholate).
2. Coat ELISA plates with 100 µl of diluted antigen per well. Cover and incubate overnight at +4 ºC.
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 (10 mM potassium phosphate, pH 7.4, 0.15 M NaCl) containing 1% BSA per well. Incubate at room temperature for three hours.
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.