β_2-GLYCOPROTEIN 1

ATG01-10 β_2-Glycoprotein 1 1.0 mg

Description of the Product

Purified from human plasma that has been tested and found to be negative for HIV, HCV and HBsAg antibodies. After coating onto ELISA plates the product will bind autoantibodies to β_2-glycoprotein 1.

Purity: The β_2-glycoprotein 1 autoantigen (54 kDa) is more than 95% pure, as assessed by SDS 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 directed to negatively charged phospholipids, in particular cardiolipin, have been detected in the serum of patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS)[1]. APS is characterised by venous and arterial thrombosis, recurrent spontaneous abortions and thrombocytopenia. It is now known that a serum cofactor, β_2-glycoprotein 1, is required for the binding of cardiolipin by autoantibodies in the sera of patients with APS[2-4]. By contrast, anticardiolipin antibodies from patients with infectious diseases (in particular, syphilis) do not require this cofactor[5].

β_2-Glycoprotein 1, also known as apolipoprotein H, is a relatively abundant serum protein (present at a concentration of about 0.2 mg/ml) that may play a role in coagulation[6]. It has been shown to bind to platelets[7], mitochondria[8] and negatively charged substances such as heparin[9], DNA[10], dextran sulphate[11] and negatively charged phospholipids[12]. β_2-Glycoprotein 1 is known to be very heat stable[2]. On SDS-electrophoresis the protein displays an apparent m.wt. of 45 kDa under non-reducing conditions and 55kDa upon reduction. Although isoelectric focussing has been reported[13] to reveal genetic polymorphisms of β_2-glycoprotein 1, the multiple bands seen by this method may be due to different amounts of sialic acid[14].

The amino acid sequence of β_2-glycoprotein 1 reveals a 326 amino acid protein of about 36 kDa[15]. This sequence has been confirmed by sequencing the protein's cDNA[16]. The fact that the protein migrates on SDS-electrophoresis with a much larger apparent m.wt. suggests that it is extensively glycosylated. β_2-Glycoprotein 1 has five consensus repeats, referred to as "Sushi domains", characteristic of the complement control protein family[17,18]. The use of purified β_2-glycoprotein 1 for the detection of autoantibodies in human serum has been demonstrated by several authors[19-34].

Methodology

The following is an ELISA procedure that can be used to detect anti β_2-glycoprotein 1 antibodies in human serum using the ATG01 purified antigen:

1. Dilute the purified antigen to 2.0-4.0 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 overnight at +4 °C.
3. Empty the plate 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. Incubate at room temperature for 3 hours.
5. Empty plates and apply 100 µl of serum samples diluted 1:100 in PBS / 1% BSA / 1% casein / 0.02% Tween® 20. Incubate at room temperature for 1 hour.
6. Empty plates and add 200 µl PBS/ 0.02% 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 / 0.02% 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.

Note: The type of ELISA plate[28,35] and the use of Tween® 20 in the wash buffer[36-38] are the subjects of some discussion.

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NOTE: **No patented technology** has been used by **AroTec** during the preparation of this product.

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