

## ATP04-10 Prothrombin 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 prothrombin.

**Purity:** The prothrombin autoantigen (95 kDa) is more than 95% pure, as assessed by SDS gel electrophoresis.

**Concentration:** 0.5-2.0 mg protein/ml.

**Storage:** The product is stabilised with 20% glycerol and 0.1% Micr-O-protect™. Store at -20 °C or below (long term) or at +4°C (short term). Avoid repeated freezing and thawing. Mix thoroughly before use.

### Clinical and Biochemical Data

Autoantibodies directed to negatively charged phospholipids, determined as anti-cardiolipin and/or lupus anticoagulant (LA), have been detected in the serum of patients with systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS)<sup>1-3</sup>. APS is characterised by high incidences of venous and/or arterial thrombosis, recurrent spontaneous abortions and thrombocytopenia. It is now known that serum cofactors are required for the binding of phospholipids by autoantibodies in the sera of patients with APS<sup>4,5</sup>. Beta2-glycoprotein 1 was the first target protein identified and autoantibodies directed against this protein are known to be strongly associated with thrombosis<sup>6,11</sup>. More recently autoantibodies to prothrombin have been implicated in LA activity<sup>12-14</sup>. The detection of antiprothrombin antibodies is now considered to be confirmatory for LA and to have a very high specificity for the diagnosis of APS<sup>15</sup>.

Prothrombin (also known as coagulation factor II or F2) is a vitamin K-dependent glycoprotein that is physiologically activated by the prothrombinase complex (activated factor X, factor V, calcium and phospholipids) via a series of proteolytic cleavages to yield  $\alpha$ -thrombin<sup>16</sup>. As well as triggering fibrinogen polymerisation into fibrin,  $\alpha$ -thrombin also acts on factors V, VIII, and XIII as well as protein C, platelets and endothelial cells<sup>17,18</sup>.

The amino acid sequence of prothrombin determined by cloning and sequencing its cDNA reveals a 622 amino acid protein of about 70 kDa<sup>19</sup>. Mature prothrombin that circulates in blood consists of a single 65 kDa polypeptide chain of 579 amino acids due to the removal of signal and propeptide sequences at the N-terminal. The fact that the protein migrates on SDS-electrophoresis with a much larger apparent molecular weight (95 kDa) suggests that it is extensively glycosylated. The protein also exhibits a cluster of 10  $\gamma$ -carboxyglutamic residues, referred to as the GLA domain, which is essential for the calcium-dependent binding of phospholipid to prothrombin<sup>20</sup>. As well as the  $\alpha$ -thrombin domain there are also 2 kringle domains that are believed to interact with factor Va during the assembly of the prothrombinase complex<sup>21</sup>. Antiprothrombin autoantibodies appear to be conformation-dependent and do not react with prothrombin in Western blot<sup>22</sup>. The use of purified prothrombin for the detection of autoantibodies in human serum has been described by several authors<sup>13,14,23-25</sup>.

### Methodology

The following is an ELISA procedure that can be used to detect anti-prothrombin antibodies in human serum using the ATP04 purified antigen:

1. Dilute the purified antigen to 2.0-4.0  $\mu$ g/ml in PBS (10 mM Potassium phosphate, pH 7.4, 0.15 M NaCl).
2. Coat gamma-irradiated ELISA plates with 100  $\mu$ l 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  $\mu$ l PBS containing 1% BSA per well. Incubate at room temperature for 3 hours.
5. Empty plates and apply 100  $\mu$ l of serum samples diluted 1:100 in PBS / 1% BSA / 0.02% Tween® 20. Incubate at room temperature for 1 hour.
6. Empty plates and add 200  $\mu$ l PBS/ 0.02% Tween® 20 per well. Incubate 5 minutes then empty plates. Repeat this step twice.
7. Apply 100  $\mu$ l anti-human IgG- or IgM-enzyme conjugate (horseradish peroxidase or alkaline phosphatase) diluted in PBS / 1% BSA / 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.

### References

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



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