PARIETAL CELL ANTIGEN (H/K-ATPase)

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ATP01-02 Parietal cell antigen 0.20 mg
ATP01-10 Parietal cell antigen 1.0 mg

Description of the Product

Purified from pig gastric mucosa. After coating onto ELISA plates the product will bind anti-parietal cell antibodies specific for the H/K-ATPase α- and β-subunits.

Purity: The H/K-ATPase α- and β-subunits (95 and 60-90 kDa) are 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

Autoimmune gastritis is an organ-specific autoimmune disease characterised by type A chronic atrophic gastritis and circulating autoantibodies to gastric parietal cells and intrinsic factor[1]. The end stage of type A chronic atrophic gastritis is pernicious anaemia, a disease which affects predominantly caucasians of north European origin and which is considered to be the most common cause of vitamin B_{12} deficiency in Western countries[2]. The prevalences of pernicious anaemia and type A chronic atrophic gastritis in Western populations are estimated to be 0.1 and 1.0 % respectively[2]. Antiparietal cell antibodies can be detected in the serum of 80-90% of pernicious anaemia patients[3].

Recent studies[4-6] have demonstrated that the major molecular targets recognised by parietal cell autoantibodies are the α- and β-subunits of the gastric proton pump (H/K-ATPase)[7]. The H/K-ATPase is the most abundant membrane protein of the canalicular and tubovesicular membranes of parietal cells. It catalyses the countertransport of H^+ and K^+ in an electroneutral fashion at the expense of ATP and is thereby responsible for the acidification of the stomach.

Although the H/K-ATPase α-subunit cDNA codes for a protein of m.wt. 114 kDa[8], it migrates on SDS-electrophoresis as a protein of m.wt. 95 kDa. On the other hand, the β-subunit migrates as a protein of 60-90 kDa, whereas its cDNA codes for a protein of 35 kDa[9]. Extensive glycosylation is presumably responsible for this phenomenon[10]. The amino acid sequence of the human H/K-ATPase is highly homologous to that of the porcine protein[8]. Of the 1,035 residues of the α-subunit, 1,015 are identical with those of the pig enzyme; of the 291 amino acids of the β-subunit, 251 are identical between the two species[9]. It has been demonstrated by several authors[11-14] that the porcine protein can serve as an effective ELISA substrate for the detection of human antiparietal cell autoantibodies.

Methodology

The following is an ELISA procedure which can be used to detect antiparietal cell antibodies in human serum using the ATP01 purified parietal cell antigen:

1. Dilute the purified antigen to 0.5-1.0 µg/ml in 10 mM Tris-HCl, pH 8.0 / 0.15 M NaCl / 0.1% sodium desoxycholate.
2. Coat ELISA plates with 100 µl of diluted antigen per well. Cover and incubate overnight at +4 ºC.
3. Empty plates and remove excess liquid by tapping on a paper towel.
4. Block excess protein binding sites by adding 200 µl PBS (phosphate buffered saline) 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.

References

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