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Product Specification Sheet

Protrhrombin (Factor II) Protein and antibodies

□ Cat. # PRTN11-AS-2 Sheep Anti-Human Prothrombin (Factor II) IgG-Sepharose **SIZE**: 2 ml

Thrombin (EC 3.4.21.5, fibrinogenase, thrombase, thrombofort, topical, thrombin-C, tropostasin, activated blood-coagulation factor II, blood-coagulation factor IIa, factor IIa, E thrombin, beta-thrombin, gamma-thrombin) is a serine protease that in humans is encoded by the F2 gene.[2][3] **Prothrombin** (coagulation factor II or **F II**) is proteolytically cleaved to form thrombin in the coagulation cascade, which ultimately results in the reduction of blood loss. Thrombin in turn acts as a serine protease that converts soluble fibrinogen into insoluble strands of fibrin, as well as catalyzing many other coagulation-related reactions. Prothrombin is produced in the liver and is post-translationally modified in a vitamin K-dependent reaction that converts ten glutamic acids on prothrombin to gamma-carboxyglutamic acid (Gla). In the presence of calcium, the Gla residues promote the binding of prothrombin to phospholipid bilayers (see the picture). Deficiency of vitamin K or administration of the anticoagulant warfarin inhibits the production of gamma-carboxyglutamic acid residues, slowing the activation of the coagulation cascade. Activation by prothrombinase occurs by sequential cleavage after residue Arg320 then after Arg271 to produce the active protease α-thrombin (37 kDa) and the byproduct prothrombin fragment 1.2 (35 kDa). The product thrombin further cleaves prothrombin fragment 1.2 after residue Arg155 into individual prothrombin fragments 1 and 2. The concentration of prothrombin in plasma is ~100 µg/ml (~1.4 µM).

The molecular weight of prothrombin is approximately 72,000 Da. The catalytic domain is released from prothrombin fragment 1.2 to create the active enzyme thrombin, which has a molecular weight of 36,000 Da. Activation of prothrombin is crucial in physiological and pathological coagulation. Various rare diseases involving prothrombin have been described (e.g., hypoprothrombinemia). Anti-prothrombin antibodies in autoimmune disease may be a factor in the formation of the lupus anticoagulant also known as (antiphospholipid syndrome). Hyperprothrombinemia can be caused by the G20210A mutation.

Source of Antibodies & Affinity matrix

Highly purified preparation of sheep anti-human prothrombin or Factor II IgG (#PRTN11-A) was coupled to Sepahrose 4B at 2-3 mg/ml IgG using CNBR method. The affinity matrix is supplied in PBS, 7.4., 0.05% azide as 50% solution (2 ml beads and 2 ml agarose).

Storage

Store at 2-4oC and never freeze the matrix.

Recommended Usage

Depletion of human Prothrombin/Factor II: Affinity column can be used for the depletion human plasma for Factor II. Depletion should be monitored by human prothrombin ELISA.

Immunoprecipitation of human Prothrombin. Use 20-50 ul suspension

Specificity & Cross-reactivity

Human Prothrombin protein (protein accession # P000734) sequence is highly conserved in various species: Chimp (99%), Pig 983%), Dog (85%), Cat (84%), Horse (81%), Cow/bovine (81%), Mouse (81%, Rat (79%), rabbit (78%), and Chicken (75%). Antibody to human prothrombin (#PRTN11-A) found to be crossreactive with mouse, rat, rabbit, pig, and dog. Other species not tested. Purified human, bovine, rabbit, and mouse prothrombin proteins are also available for control studies. Prothrombin Fragement I and 2 and Factor II deficient plasma are also available.

Recommended Usage

Anti-prothrombin IgG-Sepharose/Agarose may be used for immunoprecipitation and for affinity purification of prothrombin or its removal from serum or plasma or other biological fluids.. The binding capacity of the column for the affinity purification of the protein must also be evaluated for a given sample.

Removal or Depletion of prothrombin from serum.

The purification can be performed using a small column or the batch process. Purification can be performed at 4oC (for temp sensitive proteins) or room temp.

1. Dilute serum or plasma 1:2-1:5 with saline. Pass it over the anti-prothrombin- IgG Sepahrose column and recycle 2-3 times.
2. Collect the flow through, and wash until the OD280 is <0.020 or achieved a base line.
3. Elute proteins with 0.1M ammonium hydroxide (pH 11-12) into vials containing 30-50 ul of 1N acetic acid per ml of eluant. Collect 0.5-1 ml fraction (5-10 ml total).
4. Low pH elution (Tris-Gly pH 2.5, followed by neutralization in 1M Tris pH 8.0) can also be used.
5. Affinity column should not be exposed to low or high pH for prolonged periods of time.
6. Binding and elution buffers must be optimized for a given protein.
7. Bound proteins should be dialyzed against PBS or other buffer at 4oC and concentrated if necessary.

General References: Royle NJ (1987) Somat. Cell Mol. Genet. 13, 285-292; Degen SJ (1987) Biochem. 26, 6165-6177; Huang M (2003) Nat. Struct. Biol. 10, 751-756;

*This product is for In vitro research use only.

Related material available from ADI

Anti-human Factor II, VII, Willebrand Facotr, factor IX, factor X, Factor XIII, Protien C, Proteins S, Plasminogen, Fibrinogen, Fibrin, and anti-thrombin (ATIII).

Human Prothrombin ELISA

PRTN11-AS-2 130619A

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