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# ProductInformation

**Monoclonal Anti-Human Protein C Agarose**
Clone HC-2
Purified Immunoglobulin

Product No. **A 0435**

## Product Description

Monoclonal Anti-Human Protein C is derived from the hybridoma produced by the fusion of mouse myeloma cells (cell line Sp 2/O-Ag-14) and splenocytes from an immunized mouse. Protein C purified from human plasma was used as the immunogen. The antibody is purified by HPLC using a protein A column. The isotype is determined by a double diffusion assay using immunoglobulin and subclass specific antisera. Purified monoclonal anti-human protein C is coupled to cyanogen bromide-activated agarose, at 2-2.5 mg antibody per ml bed volume.

Monoclonal Anti-Human Protein C, a divalent cation independent antibody, recognizes an epitope on the heavy chain of protein C and binds to protein C zymogen. The antibody strongly inhibits the activation of protein C but has no effect on the function of activated protein C. No reaction is observed with the activation peptide itself or with the heavy chain after removal of the activation peptide.

Assays of plasma protein C levels are useful for the detection of hereditary and acquired deficiency states as well as for studies of the control mechanisms of blood coagulation and fibrinolysis. Monoclonal Anti-Protein C Agarose has proved to be a valuable tool for use in immunoaffinity purification of human Protein C and in the preparation of Protein C depleted plasma for coagulation research.

Protein C, a vitamin K dependent plasma zymogen, plays an essential role in the regulation of blood coagulation. The nucleotide sequence of the gene that codes for protein C has been determined.[1] Protein C is synthesized by liver parenchymal cells as a single chain polypeptide,[2] but in plasma it consists mainly of a heavy chain (41 kDa) linked by a disulfide bond to a light chain (21 kDa).[3] The plasma concentration of protein C is approximately 4 µg/ml with a half-life of about 15 hours.[4]

Activation of human protein C involves the release of a dodeca-peptide from the C-terminal domain of the heavy chain.[1] This is accomplished inefficiently by thrombin, which cleaves an Arg-Leu bond, but when thrombin forms a 1:1 high affinity complex with the endothelial membrane protein thrombomodulin, activation of protein C is accelerated approximately 20,000 fold.[5] Activated protein C cleaves essential peptide bonds in the heavy chains of factors Va and VIIIa which result in their inactivation and consequently in inhibition of the coagulation cascade.[6, 7, 8] Free plasma protein S serves as a cofactor for activated protein C's inhibitory functions, probably by enabling the reactions to take place on platelet and endothelial cell membranes.[5] Activated protein C also enhances fibrinolysis by forming a complex with plasminogen activator inhibitor, thus allowing enhanced activity of plasminogen activator.[9] Inactivation of activated protein C in plasma requires at least two "serpin" inhibitors. One inhibitor's activity is enhanced by heparin[10] while the other (α-1-antitrypsin) is heparin independent.[11]

Hereditary and acquired protein C deficiency states have been recognized to be associated with thrombosis. Homozygous severe protein C deficiency manifests in the newborn by massive thrombosis[12] and purpura fulminans.[13] Heterozygotes, for this entity usually do not manifest thrombosis.[14, 15] However, patients affected by a different heterozygous (partial) protein C deficiency frequently present a thrombotic tendency during young adulthood.[16] Acquired deficiency has been observed in patients with disseminated intravascular coagulation, liver diseases, complications following surgery and in those taking coumarin drugs.[17]

## Reagent

Monoclonal Anti-Human Protein C Agarose is supplied as a 1:1 suspension in 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 0.1% sodium azide as a preservative.

**Precautions and Disclaimer**
Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

**Storage/Stability**
Monoclonal Anti-Human Protein C-agarose may be regenerated and used for future adsorptions. Strip the agarose with ten column volumes of 0.1 M glycine, 0.15 M sodium chloride, pH 2.4, or 0.5 M acetic acid, 0.15 M sodium chloride, pH 2.4, then wash with 0.01 M sodium phosphate buffer, pH 7.2, containing 0.5 M sodium chloride (PB). Regenerated agarose may be stored at 2-8 °C as a suspension in PB containing preservative. Do not freeze.

**Binding Capacity**
One milliliter of adsorbent depletes at least 20 milliliters citrated normal human plasma from Protein C at 99.9% efficiency (at flow rate of 300 µl/min.). Depletion is measured by ELISA using Monoclonal Anti-Human Protein C, Clone HC-2 (Product Code P 5305) and Alkaline Phosphatase conjugated Monoclonal Anti-Human Protein C, Clone HC-4 (Product Code A 9683). Coagulation activity of the depleted plasma remains within normal values.

**References**

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