

# Product No. P-5305 Lot 037H4822

### Monoclonal Anti-Human Protein C

Purified Mouse IgG1 Clone HC-2

Monoclonal Anti-Human Protein C (mouse IgG1 isotype) 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 using Sigma ImmunoType<sup>TM</sup> Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is supplied as a liquid in 10mM HEPES, 140mM NaCl, pH 7.4, containing 0.05% sodium azide (see MSD\$\frac{S}{2}\$) as a preservative.

# Specificity

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.

## **Description**

Protein C is a vitamin K dependent plasma zymogen which plays an essential role in the regulation of blood coagulation. The nucleotide sequence of the gene that codes for protein C has been determined<sup>1</sup>. Protein C is synthesized by liver parenchymal cells as a single chain polypeptide<sup>2</sup>, but in plasma it consists mainly of a heavy chain (41 kD) linked by a disulfide bond to a light chain  $(21 \text{ kD})^3$ . The plasma concentration of protein C is approximately 4  $\mu$ g/ml with a half-life of about 15 hours<sup>4</sup>. Activation of human protein C involves the release of a dodeca-peptide from the C-terminal domain of the heavy chain<sup>1</sup>. 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 thrombo-modulin, activation of protein C is accelerated approximately 20,000 fold<sup>5</sup>. Activated protein C cleaves essential peptide bonds in the heavy chains of factors Va and VIIIa which result in their in-activation and consequently in inhibition of the coagulation cascade<sup>6,7,8</sup>. 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<sup>5</sup>. Activated protein C also enhances fibrinolysis by forming a complex with plasminogen activator inhibitor, thus allowing enhanced activity of plasminogen activator9. Inactivation of activated protein C in plasma requires at least two "serpin" inhibitors. One inhibitor's activity is enhanced by heparin<sup>10</sup> while the other ( $\alpha$ -1-antitrypsin) is heparin independent<sup>11</sup>.

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<sup>12</sup> and purpura fulminans<sup>13</sup>. Heterozygotes for this entity usually do not manifest thrombosis<sup>14,15</sup>. However, patients affected by a different heterozygous (partial) protein C deficiency frequently present a thrombotic tendency during young adulthood<sup>16</sup>. Acquired deficiency has been observed in patients with disseminated intravascular coagulation, liver diseases, complications following surgery and in those taking coumarin drugs<sup>17</sup>.

#### Uses

Anti-Protein C may be used for immunochemical determination of protein C levels in normal and pathogenic human plasma. Determination of protein C levels can be used in the study of regulation of blood coagulation and fibrinolysis.

**Protein Concentration:** 4.0 mg/ml

**Working Dilution** 

A dilution of 1:400 as determined by indirect immunoblotting using denatured and reduced pooled plasma.

In order to obtain best results it is recommended that each individual user determine their working dilution by titration assay.

## Storage

For continuous use, store at 2-8°C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

\*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.

## References

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