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monoclonal antibody against human γ -carboxyglutamyl (Gla) residues Product No. 3570

Description

A murine IgG_{2b} monoclonal antibody directed against γ -carboxyglutamyl (Gla) residues found in human coagulation proteins. The antibody has been grown in ascites and purified via Protein G affinity chromatography.

Properties

No. 3570 reacts with the gla domains found in human prothrombin, prothrombin fragment 1, factor VII, recombinant factor VIIa, factor IX, factor X, Protein C, Protein S and bovine bone Gla protein. Ca⁺² (5 mM concentration) strongly inhibits the binding of No. 3570 to human prothrombin in a non-competitive immunofluorescence assay.

Applications

- A. **Western Blot** – Following SDS-PAGE and electrophoretic transfer of the protein to a membrane, incubate at 5 μ g/mL for 1 hour at room temperature. No. 3570 recognizes the protein listed under reducing and denaturing conditions. If the protein is a two-chain protein, such as FVIIa, FIXa, FX and Protein C, only the light-chain containing the Gla residue is recognized.
- B. **Immunopurification of Gla-containing Proteins** – When linked to a solid-phase support, No. 3570 can be used for immunopurification of Gla-containing proteins utilizing a mild 50 mM Ca⁺² elution process.

Presentation

0.5 mg of purified antibody lyophilized from a solution of 0.5 mL of 0.15M Phosphate Buffered Saline, 0.2M mannitol, pH 7.4.

Reconstitution

Add 0.5 mL of distilled water for 1.0 mg/mL stock solution.

Storage

Store lyophilized antibody at 2° - 8°C. Store reconstituted antibody at -20°C or colder.

References

Brown, M. A., *et al.* *Journal of Biological Chemistry* 2000, **275**: 19795-19802.

matrix bound monoclonal antibody against human γ -carboxyglutamyl (Gla) residues

Product No. 3570Mx

Description

Product No. 3570 (for description, see the reverse side), a monoclonal antibody directed against γ -carboxyglutamyl (Gla) residues found in human coagulation proteins, bound to Sepharose CL-4B for use in the affinity purification of Gla-containing proteins of human and other origins such as prothrombin, factor VII/VIIa, factor IX/IXa, factor X/Xa, Protein C, Protein S and growth arrest-specific protein-6 (Gas6).

Properties

The antibody is bound to the support at a concentration of 5 mg of antibody/mL of settled gel.

Purification Protocol

Using the following buffers, a suggested purification protocol is,

Loading Buffer:	20 mM Tris-HCl, 150 mM NaCl, 10 mM EDTA, pH 7.4
Washing Buffer:	20 mM Tris-HCL, 350 mM NaCl, 10 mM EDTA, pH 7.4
Elution Buffer:	20 mM Tris-HCl, 150 NaCl, 50 mM CaCl ₂ , pH 7.4

1. Pack the gel in a suitable column, approximately 16 mm in diameter
2. Equilibrate the column with Loading Buffer at a flow rate of 2 mL/min.
3. Load the sample to be purified using a flow rate of 0.5 mL/min.
4. Wash the column with Washing Buffer using a flow rate of 2 mL/min.
5. Once the A₂₈₀ of the effluent has decreased to the baseline, elute the bound protein with Elution Buffer using a flow rate of 2 mL/min.
6. Dialyze the elution fraction against a suitable buffer (e.g. TBS, Tris Buffered Saline, pH 7.4) and store at -20°C.

Note: As Ca⁺² ions in the sample may inhibit binding, the presence of a chelator such as EDTA (2-10 mM) is recommended during the binding step. In some cases, Ca⁺² concentrations >50 mM may enhance the elution step.

Presentation

1 mL of settled gel immobilized with 5 mg of purified antibody in a buffer of 20 mM Tris-HCl, 500 mM NaCl, pH 7.4 with 0.02% NaN₃ added as a preservative.

Storage

Store the immobilized gel at 2° - 8°C.

References

- Brown, M. A., *et al.* *Journal of Biological Chemistry* 2000, **275**: 19795-19802.
Stenberg, L. M. *et al.* *Biochemical and Biophysical Research Communications* 2001, **280**: 1036-1041.
Stenberg, L. M. *et al.* *Biochemical and Biophysical Research Communications* 2001, **283**: 454-459.