



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sia.com
sigma-aldrich.com

Product Information

Monoclonal Anti-Endothelial Cell Protein C Receptor (EPCR)

Clone RCR-252
Purified Rat Immunoglobulin

Product Number **E 6280**

Product Description

Monoclonal Anti-Endothelial Cell Protein C Receptor (EPCR) (rat IgG1 isotype) is derived from the RCR-252 hybridoma produced by the fusion of mouse SP2/0 myeloma cells and cells isolated from the superficial inguinal lymph nodes from Wister rats immunized with human EPCR-positive RE-1 cells.¹ The antibody is purified from culture supernatant of hybridoma cells, grown in a bioreactor.

Monoclonal Anti-Endothelial Cell Protein C Receptor (EPCR) recognizes human EPCR (49 kDa). The antibody may be used in flow cytometry (FACS analysis)¹ and in blocking the binding of the APC ligand to the EPCR.^{1,2}

Blood coagulation depends on a series of sequential proteolytic processes, leading to the generation of new serine proteases. Several natural anticoagulant mechanisms are balancing the clotting system. One of them is the protein C anticoagulant pathway. In this pathway, thrombin binds to the endothelial cell receptor thrombomodulin. This complex activates protein C to generate the anticoagulant enzyme activated protein C (APC) that when complexed to protein S inhibits coagulation by inactivating two critical regulatory proteins: factor Va and VIIIa.³

Protein C activation by the thrombin/ thrombomodulin complex is a relatively low-affinity reaction. However, it was found that protein C and APC bind to culture endothelial cells with a relatively high affinity. The high affinity binding of both ligands ($K_d = 30\text{nM}$) is mediated by EPCR (endothelial cell protein C receptor). EPCR is a type 1 transmembrane glycoprotein containing two domains in the extra cellular region that are homologous to the $\alpha 1$ and $\alpha 2$ domains of CD1/MHC class 1 molecules.⁴ The mature human EPCR protein contains 211 amino acids.

The protein migrates as a 49 kDa protein due to its carbohydrates chains.¹ The protein is expressed in endothelial cells present in various types of vessels including arterial heart, lung, and in the vena cava inferior. The EPCR is also expressed in small vessels such as capillaries of the alveolar wall in the lung.¹

The high-affinity binding of protein C to EPCR is a critical step for activation. Upon binding, the structure of protein C is modulated to enable rapid conversion to the active form. This enhances the activation rate by at least 5-fold.^{3,4} The EPCR protein appears to perform other functions, as well. It binds proteinase 3. The complex then binds to MAC-1 on activated neutrophils and inhibits their adhesion to activated endothelium. Another property of EPCR is that it can translocate from the membrane to the nucleus.⁵

The RCR-252 monoclonal antibody strongly inhibits the interaction of APC with EPCR. The region recognized by the RCR-252 antibody is critical for binding of APC.² Antibodies, which react specifically with the EPCR protein and inhibit ligands binding to it, are an essential tool for exploring the physiological effects of protein C and APC binding on the mechanisms of blood clotting in homeostasis and various thrombotic disorders.

Reagent

Monoclonal Anti-Endothelial Cell Protein C Receptor (EPCR) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

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

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

A working concentration of 5-20 µg/ml is recommended by FACS analysis (flow cytometry) using HUVEC cells.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working concentrations by titration.

References

1. Ye, X., et al., *Biochem. Biophys. Res. Commun.*, **259**, 671-677 (1999).
2. Shu, F., et al., *FEBS Lett.*, **477**, 208-212 (2000).
3. Stearns-Kurosawa, D.J., et al., *Proc. Natl. Acad. Sci. USA*, **93**, 10212-10216 (1996).
4. Fukudome, K., et al., *J. Exp. Med.*, **187**, 1029-1035 (1998).
5. Oganeyn, V., et al., *J. Biol. Chem.*, **277**, 24851-24854 (2002).

KAA/EK 01/04

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.