Anti-human VEGFR-2/KDR (#4 (20I6))

20141001BB

FOR RESEARCH ONLY! NOT FOR HUMAN USE!

Cat.-no.:	**101-M34**
Size:	100 µg
Lot. No.:	According to product label
Country of origin:	Germany

Preparation: Monoclonal antibodies were produced with the help of BALB/c mice using recombinant human soluble extracellular KDR (D7) as the immunizing antigen

Target Background

Synonyms:	Vascular endothelial growth factor receptor 2

Disruption of the precise balance of positive and negative molecular regulators of blood and lymphatic vessel growth can lead to myriad diseases. Although dozens of natural inhibitors of hemangiogenesis have been identified, an endogenous selective inhibitor of lymphatic vessel growth has not to our knowledge been previously described. A splice variant of the gene encoding vascular endothelial growth factor receptor-2 (VEGFR-2) that encodes a secreted form of the protein, designated endogenous soluble VEGFR-2 (esVEGFR-2/KDR) has been described. The endogenous soluble esKDR inhibits developmental and reparative lymphangiogenesis by blocking VEGF-C function. Tissue-specific loss of esKDR in mice induced, at birth, spontaneous lymphatic invasion of the normally alymphatic cornea and hyperplasia of skin lymphatics without affecting blood vasculature. Administration of esKDR inhibited lymphangiogenesis but not hemangiogenesis induced by corneal suture injury or transplantation, enhanced corneal allograft survival and suppressed lymphangioma cellular proliferation. Naturally occurring esKDR thus acts as a molecular uncoupler of blood and lymphatic vessels; modulation of esKDR might have therapeutic effects in treating lymphatic vascular malformations, transplantation rejection and, potentially, tumor lymphangiogenesis and lymphedema.

References

1. Shibata et al, BMC Medicine 8 (2010)
2. Albaquerque et al, Nature Med 2009
3. Ebos et al, Mol Cancer Res 2 (2004)
4. Ebos et al, Cancer res 68 (2008).
5. Benzinger et al., BBA 1466:71, 2000

Database References Antigen

Protein RefSeq:	NP_002241.1
Uniprot ID:	P22333
mRNA RefSeq:	NM_002253.2

Product Specifications

Species reactivity	human
Clone/Ab feature	IgG_1; #4 (20I6)
Cross reactivity	ND
Host	mouse
Clonality	monoclonal
Purification	Protein G purified
Immunogen	recombinant human soluble KDR (D7) (RT# S01-003)
Formulation	lyophilized
Buffer	PBS

Stability: The lyophilized antibody is stable at room temperature for up to 1 month. The reconstituted antibody is stable for at least two weeks at 2-8°C. Frozen aliquots are stable for at least 6 months when stored at -20°C.

Reconstitution: Centrifuge vial prior to opening. Reconstitute in sterile water to a concentration of 0.1-1.0 mg/ml.

AVOID REPEATED FREEZE AND THAW CYCLES!

Specificity: The antibody will detect native human VEGFR-2/KDR in ELISA and on the surface of different human cell types.

Applications

Western Blot:	Use at 2-5 µg/ml
FACS:	Use at 2-5 µg/ml
IF/IHC	IHC: 6-30 µg/ml; IF: 2-10µg/ml
ELISA:	Use at 1-10 µg/ml

NOTE: OPTIMAL DILUTIONS SHOULD BE DETERMINED BY EACH LABORATORY FOR EACH APPLICATION!

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Handling/Applications

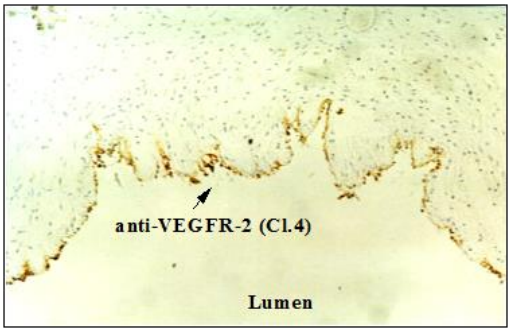

Figure 1: Up-regulation of VEGFR-2 in vein ECs of an intact human umbilical cord by bFGF. A fresh human umbilical cord was rinsed with PBS to remove residual blood cells, cut in small pieces (about 0.5 cm), incubated in EBM (1% FCS) and stimulated with or without 20 ng/ml bFGF for 24 h. Pieces were frozen in liquid nitrogen and used for immunohistochemistry using the mab anti-human VEGFR-2/Cl.4 **(#101-M34)** as detection antibody. (Bernhard Barleon et al., unpublished data!)

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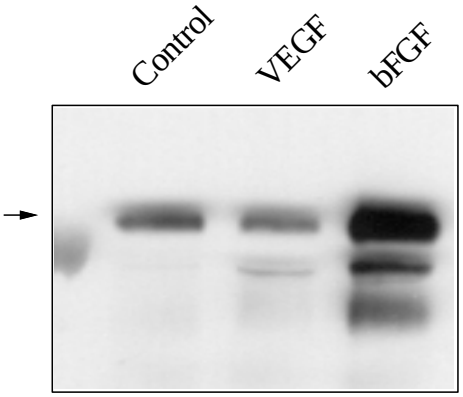

Figure 2: Up-regulation of VEGFR-2 in primary HUVECs by bFGF. Freshly isolated HUVECs (passage 1) were cultured in EBM. Subconfluent cultures were stimulated with VEGF (5 ng/ml) or bFGF (10 ng/ml) for 3 days. Total lysate was prepared and subjected to immunoprecipitation (anti-human VEGFR-2 (Cl.3) **[#101-M32**] followed by Western blotting (anti-human VEGFR-2 (Cl.4) **[#101-M34**]. (Bernhard Barleon et.al., unpublished data!)

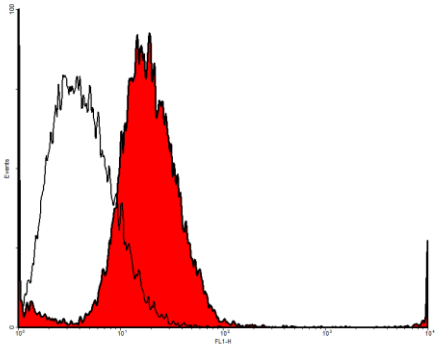

Figure 3. FACS analysis with primary human dermal lymphatic endothelial cells (HDLEC).

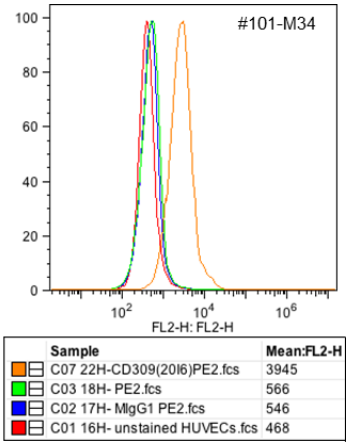

Figure 4: FACS analysis of VEGFR-2/KDR expression in HUVE cells [5µg/ml #101-M32; 5µg/ml PE goat anti-mouse IgG].
The experiment was performed by Trisha M. Westerhof, University of California, Irvine.

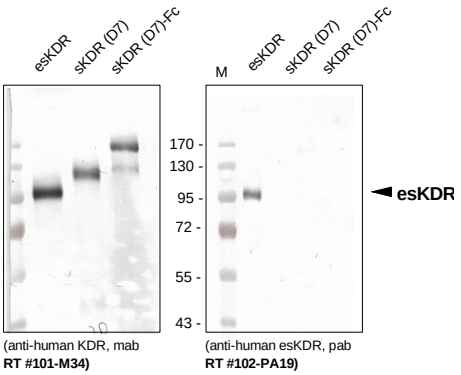

Figure 5: Western Analysis of anti-human esKDR. Samples were loaded in 10% SDS-polyacrylamide gel under reducing conditions. Left panel: monoclonal antibody against the soluble KDR (D1-7); Right panel: polyclonal antibody (peptide) against the unique C-terminal end of esKDR.

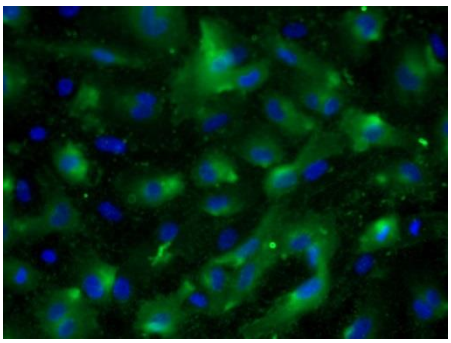

Figure 6: Immunofluorescence staining (green) of VEGFR-2 (KDR) in primary human umbilical vein endothelial cells (HUVEC) with anti-human VEGFR-2 (Cl.4) (7,5µg/ml) [**Cat# 101-M34**] and counter staining of nuclei with Dapi. As secondary antibody goat anti-mouse ALEXA Flour 488 (Dianova) was used 1:600.