Recombinant Rat Vascular Endothelial Growth Factor-C_{152S}

20180216BB

FOR RESEARCH ONLY! NOT FOR HUMAN USE!

Cat.-no:	**R20-017**
Size:	20 µg
Lot. No.:	According to product label
Country of origin:	Germany

Scientific Background

Gene:	*vegf*
Synonyms:	vascular endothelial growth factor C; Vegfc

VEGF-C_{152S} is a point mutant generated by the replacement of the second conserved Cys residue of the recombinant processed VEGF-C by a Ser residue. VEGF-C_{152S} is analog to the human VEGF-C_{156S} mutant and only active toward VEGFR-3/FLT-4 but, unlike wild type VEGF-C, is unable to bind to and to activate signalling through VEGFR-2/KDR. VEGF-C_{152S} was inactive in the vascular permeability assay and did not increase migration of the capillary endothelial cells, indicating that these VEGF-like effects of VEGF-C require VEGFR-2 binding. VEGF-C, also known as Vascular Endothelial Growth Factor Related Protein (VRP), is a recently discovered VEGF growth factor family member that is most closely related to VEGF-D. The rat VEGF-C cDNA encodes a pre-pro-protein of 416 amino acids residues. It is almost identical to the mouse VEGF-C protein. Similar to VEGF-D, VEGF-C has a VEGF homology domain spanning the middle third of the precursor molecule and long N- and C-terminal extensions. Recombinant rat VEGF-C, lacking the N- and C-terminal extensions and containing only the middle VEGF homology domain, forms primarily non-covalently linked dimers. This protein is a ligand for both VEGFR-2/KDR and VEGFR-3/FLT-4. Since VEGFR-3 is strongly expressed in lymphatic endothelial cells, it has been postulated that VEGF-C is involved in the regulation of the growth and/or differentiation of lymphatic endothelium. Although recombinant rat VEGF-C is also a mitogen for vascular endothelial cells, it is much less potent than VEGF-A. The recombinant rat VEGF-C contains 129 amino acids residues and was fused to a His-tag (6x His) at the C-terminal end. As a result of glycosylation VEGF-C migrates as an 18-24 kDa protein in SDS-PAGE under reducing conditions.

References

1. Henri O et al., Circulation, March 2016
2. Joukov et al., J Biol Chem 273 :6599, 1998
3. Joukov et al., EMBO J 15:290, 1996
4. Olofsson et al., Curr Opin Biotech 10:528, 1999
5. Kirkin et al., Eur J Biochem 268:5530, 2001

Sequence

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DTVKLAAAHYNTEILKSIDNEWRKTQCMPREVCIDVGKEFGAATNTFFKPPS
VSVYRCGGCCNSEGLQCMNTSTGYLSKTLFEITVPLSQGPKPVTISFANHTS
CRCMSKLDVYRQVHSIIHHHHHH
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Database References

Protein RefSeq:	NP_446105.1
Uniprot ID:	O35757
mRNA RefSeq:	NM_053653.1

Product Specifications

Expressed in	Insect cells
Purity	> 90% by SDS-PAGE & silver stain
Buffer	50 mM acetic acid
Stabilizer	BSA
Formulation	lyophilized
Length (aa):	127
MW:	18-24 kDa
Result by N-terminal sequencing	UNDER WORK!

Stability: Lyophilized samples are stable for greater than six months at –20°C to –70°C. Reconstituted VEGF-C_{152S} should be stored in working aliquots at -20°C.

Reconstitution: Centrifuge the vial prior to opening! The lyophilized VEGF-C_{152S} should be reconstituted in PBS or medium to a concentration not lower than 50 µg/ml.

AVOID REPEATED FREEZE AND THAW CYCLES!

Biological Activity: (A) The proliferative response to rrVEGF-C_{C152S} was assayed in VEGFR3-expressing porcine aortic endothelial (PAE) cells (*in vitro*). (B) The lymphangiogenic response to rrVEGF-C_{C152S} loaded in a biopolymeric albumin-alginate microcapsules for targeted slow-release was assayed in male Wistar rats.

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Handling/Application

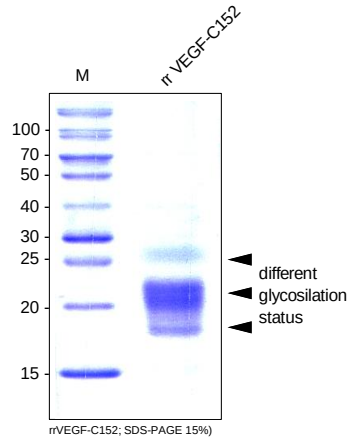

Fig. 1: SDS-PAGE analysis of recombinant rat VEGF-C_{152} mutant. Sample was loaded in 15% SDS-polyacrylamide gel under reducing conditions and stained with Coomassie blue.

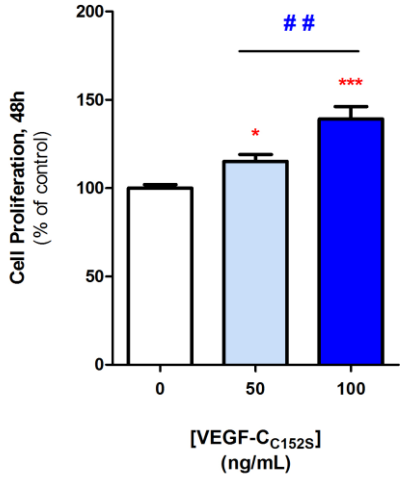

Fig. 2: *In vitro*: The proliferative response to recombinant rat VEGF-C_{C152S} was assayed in VEGFR3-expressing porcine aortic endothelial (PAE) cells. Briefly, cells were plated in 12-well plates and incubated in DMEM medium supplemented with 1% fetal calf serum for cell cycle arrest 24h prior to stimulation of cell proliferation with recombinant VEGF-C_{C152S} at the concentrations of 50 and 100 ng/mL. After 48h in culture, the cell proliferative response was assayed (WST-1 colorimetric assay), and the results expressed as % of control non-stimulated cells (mean ± sem).

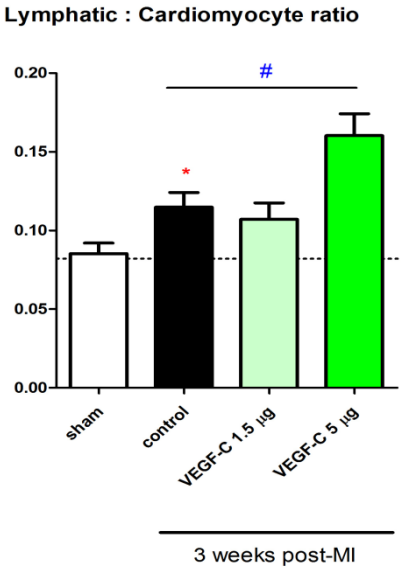

Fig. 2: *In vivo*: The lymphangiogenic response to recombinant rat VEGF-C_{C152S} loaded in our biopolymeric albumin-alginate microcapsules for targeted slow-release was assayed in male Wistar rats. Briefly, after ischemia-reperfusion injury to induce myocardial infarction, VEGF-C_{C152S} at the dose of 1.5 or 5 µg per heart was injected intra-myocardially. Lymphatic responses in the myocardium were analyzed by IHC at 3 weeks post-MI using LYVE1 antibody (RT, #103-PA50). Results are expressed as lymphatic vessel to cardiomyocyte ratio (mean ± sem).

The experiments were performed by the research group of Prof. Dr. E. Brakenhielm – *Rouen University (see also: Henri O et al., Circulation, March 2016, DOI: 10.1161)*

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