

Datasheet for 400-001-MT5**Recombinant Anti-VEGF RTH258 scFv Antibody****Overview**

Description:	Humanized Recombinant Anti-Human VEGF RTH258 scFv Antibody - 400-001-MT5
Item No.:	400-001-MT5
Size:	50 µg
Applications:	ELISA, FC, WB
Reactivity:	Human
Expressed in:	HEK293

Product Details**Background:**

VEGF is a potent mitogen in embryonic and somatic angiogenesis with specificity for vascular endothelial cells. VEGF forms homodimers and exists in four different isoforms. Overall, the VEGF monomer resembles that of PDGF, but its N-terminal segment is helical rather than extended. VEGF shares homologies of about 21% and 24% respectively with the A and B chains of human platelet-derived growth factor (PDGF), and has complete conservation of the eight cysteine residues found in both mature PDGF chains. The cysteine knot motif is a common feature of this domain. The homology is not reflected in function, however, since the cell types responsive to VEGF are distinct from those responsive to homo- and heterodimers of the PDGF chains. This protein is a glycosylated mitogen that acts on endothelial cells and has various effects, including mediating increased vascular permeability, inducing angiogenesis, vasculogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis. VEGF-A also has been shown to have effects on a number of other cell types (e.g. stimulation of monocyte/macrophage migration, neurons, cancer cells, kidney epithelial cells). VEGF-A is also a vasodilator; it increases microvascular permeability, and was originally referred to as vascular permeability factor. Alternatively spliced transcript variants, encoding either freely secreted or cell-associated isoforms, have been characterized. Antibody against VEGF-A (Bevacizumab or brand name Avastin) is approved for treatment of cancer by inhibiting angiogenesis. Ranibizumab, a Fab fragment of Avastin or so-called Lucentis is approved to treat the "wet" type of age-related macular degeneration (AMD, also ARMD), diabetic retinopathy, and macular edema due to branch retinal vein occlusion or central retinal vein occlusion. This antibody has its amino acid sequence like one of Brolucizumab (sold under trade names Beovu or Vsiqq) - a humanized single-chain antibody fragment for the treatment of neovascular (wet) age-related macular degeneration (AMD).

Synonyms:	Anti-VEGF-A Antibody, RTH258, Brolucizumab, Vascular endothelial growth factor A, VEGF A, VEGFA, Vascular permeability factor, VPF
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Expressed in:	HEK293
Clonality:	Recombinant Monoclonal
Clone ID:	Abz5
Format:	IgG

Target Details

Gene Name:	VEGF-A
Reactivity:	Human
Immunogen Type:	Recombinant Protein
Purity/Specificity:	Humanized Recombinant anti-VEGFA ScFV fragment was expressed in HEK293 cells. Purified by one-step purification by HisTrap FF Crude. The purity was estimated to be >90% by SDS-PAGE analysis.
Relevant Links:	• UniProtKB - P15692 • NCBI - NP_001020537.2 • GeneID - 7422

Application Details

Tested Applications:	ELISA, FC, WB
Application Note:	Humanized Recombinant Anti-VEGFA ScFV fragment Antibody has been tested for use in Flow Cytometry, Western Blot, and ELISA. This antibody recognizes structured VEGF-A and will work in western blot when the protein has not been denatured with DTT or bMe. Although not tested, this antibody could be useful in in IHC and in in-vivo and other cellular assays. Specific conditions for reactivity should be optimized by the end user
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	User Optimized
FC:	User Optimized
WB:	User Optimized

Formulation

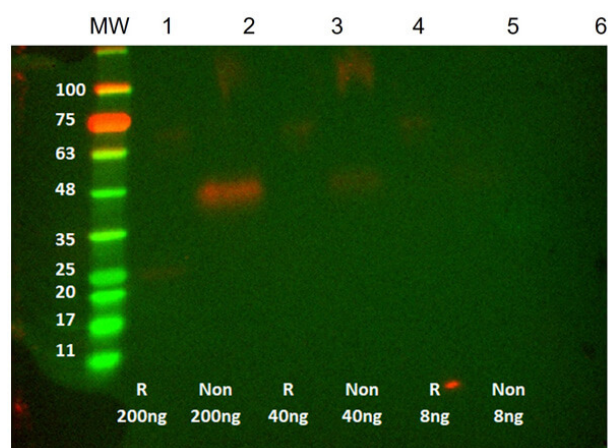
Physical State:	Liquid (sterile filtered)
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Concentration:	0.41mg/ml
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	None
Stabilizer:	30% Glycerol

Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store vial at -20° C. Dilute only prior to immediate use.
Expiration:	Expiration date is six (6) months from date of receipt.

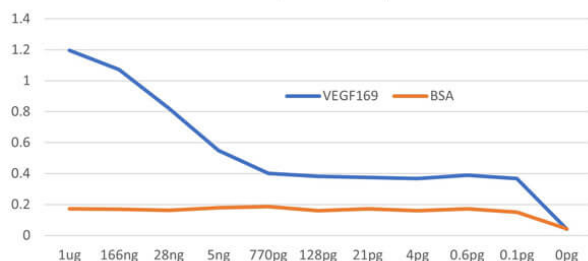
Images



Western Blot

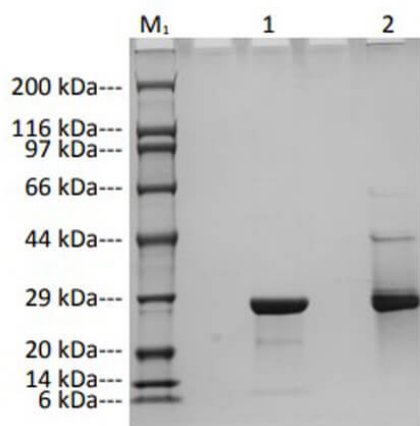
Western Blot using Humanized Recombinant Brolucizumab/RTH258 Anti-human VEGF ScFv to detect structured and denatured human VEGF165 protein. MW: Molecular Weight Ladder. Lanes 1,3, and 5: VEGF165 reduced. Lanes 2, 4, and 6: VEGF165 non-reduced. Protein Load: Lane 1-2: 200ng. Lane 3-4: 40ng. Lane 5-6: 8ng. Primary Antibody: FLAG-tagged Humanized Recombinant Brolucizumab/RTH258 Anti-human VEGF ScFv at 1.0µg/mL for 2 hours at 4°C. Secondary Antibody: Antibody for the detection of FLAG™ conjugated proteins (MOUSE) Monoclonal Antibody Peroxidase Conjugated (p/n 200-303-383) at 1:5000 for 2 hours at 4°C. The VEGF165 protein has a calculated MW of 19.2kDa. As a result of glycosylation, the protein migrates as 24kDa (monomer) under reducing (R) condition, and 43-50kDa (homodimer) under non-reducing (NR) condition (SDS-PAGE).

Anti-VEGF (RHT258) ELISA



ELISA

ELISA results show the sensitivity of Humanized Recombinant Brolicizumab/RTH258 Anti-human VEGF ScFv fragment Antibody tested against purified human VEGF165 protein. Each well was coated in duplicate with 100ng of VEGF or BSA. The starting dilution of ScFv antibody was 1µg/well with 6-fold dilution. The secondary Antibody for the detection of FLAG™ conjugated proteins (MOUSE) Monoclonal Antibody Peroxidase Conjugated (p/n 200-303-383) with 1:5,000 dilution was used to detect bound ScFv.



SDS-PAGE

SDS-PAGE of Humanized Recombinant Anti-human VEGF scFv Fab fragment Antibody. Lane M1: Molecular Weight Ladder. Lane 1: recombinant Anti-human VEGF scFv Fab fragment [reduced]. Lane 2: recombinant Anti-human VEGF scFv Fab fragment [non-reduced]. Coomassie Stained.

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.