

**Datasheet for 400-001-NG6****Recombinant Human VEGF receptor****Overview**

|                      |                                                         |
|----------------------|---------------------------------------------------------|
| <b>Description:</b>  | Humanized Recombinant Human VEGF receptor - 400-001-NG6 |
| <b>Item No.:</b>     | 400-001-NG6                                             |
| <b>Size:</b>         | 50 µg                                                   |
| <b>Applications:</b> | ELISA, SDS-PAGE, WB                                     |
| <b>Reactivity:</b>   | Human                                                   |
| <b>Expressed in:</b> | CHO cells                                               |

**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.

Aflibercept, known as EYLEA or ZALTRAP, is a recombinant fusion protein, consisting of portions of human VEGF receptors 1 and 2 extracellular domains fused to the Fc portion of human IgG1 and formulated as an iso-osmotic solution for intravitreal administration. Aflibercept acts as a soluble decoy receptor that binds VEGF-A and placental growth factor (PlGF) and thereby can inhibit the binding and activation of their cognate VEGF receptors. Aflibercept is a medication used to treat wet macular degeneration and metastatic colorectal cancer.

|                  |                                                                                                                                   |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| <b>Synonyms:</b> | Biosimilar of Aflibercept, Eylea, Zaltrap, Vascular endothelial growth factor A, VEGF A, VEGFA, Vascular permeability factor, VPF |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------|

|                      |                        |
|----------------------|------------------------|
| <b>Expressed in:</b> | CHO cells              |
| <b>Clonality:</b>    | Recombinant Monoclonal |
| <b>Clone ID:</b>     | Abz774                 |
| <b>Format:</b>       | IgG                    |

## Target Details

|                            |                                                                                                                                             |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Reactivity:</b>         | Human                                                                                                                                       |
| <b>Immunogen Type:</b>     | Recombinant Protein                                                                                                                         |
| <b>Purity/Specificity:</b> | Recombinant VEGF receptor, Biosimilar of Aflibercept, was expressed in CHO cells. The purity was estimated to be >90% by SDS-PAGE analysis. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"><li>• <a href="#">UniProtKB - P15692</a></li><li>• <a href="#">NCBI - P15692.2</a></li></ul>              |

## Application Details

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b> | ELISA, SDS-PAGE, WB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Application Note:</b>    | Humanized Recombinant Human VEGF receptor has been tested for use in Western Blot and ELISA. This recombinant receptor recognizes structured VEGF-A and will work in western blot when the protein has not been denatured with DTT or bMe. This receptor is also cross-reactive with respective rabbit and murine VEGF. Although not tested, this receptor could be useful in Flow Cytometry and IHC and in in-vivo and other cellular assays. Specific conditions for reactivity should be optimized by the end user. |
| <b>Assay Dilutions:</b>     | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>ELISA:</b>               | User Optimized                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>WB:</b>                  | 1µg/mL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

## Formulation

|                        |                                                            |
|------------------------|------------------------------------------------------------|
| <b>Physical State:</b> | Liquid (sterile filtered)                                  |
| <b>Concentration:</b>  | 1.0 mg/mL by UV absorbance at 280 nm                       |
| <b>Buffer:</b>         | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 |
| <b>Preservative:</b>   | 0.01% (w/v) Sodium Azide                                   |

Stabilizer: None

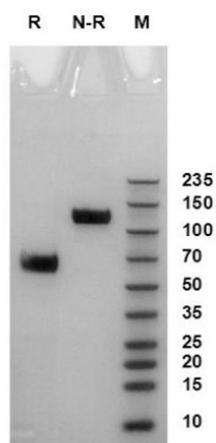
## Shipping & Handling

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



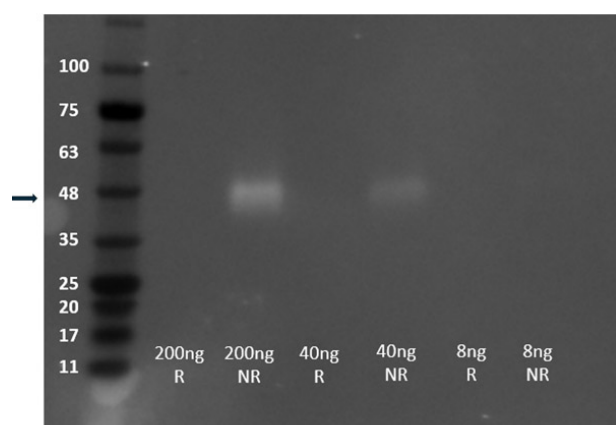
### SDS-PAGE

SDS-PAGE results of Recombinant Human VEGF receptor, Biosimilar of Aflibercept.

Lane 1 (R): reduced recombinant Aflibercept.

Lane 2 (N-R): non-reduced recombinant Aflibercept.

Lane 3 (M): Molecular Weight Ladder.



### Western Blot

Western Blot using Recombinant VEGF receptor, Biosimilar of Aflibercept (EYLEA) to detect human VEGF165 protein.

Lane 1: Molecular Weight Ladder (p/n MB-250-0500).

Lanes 2, 4, and 6 (R): VEGF165 reduced.

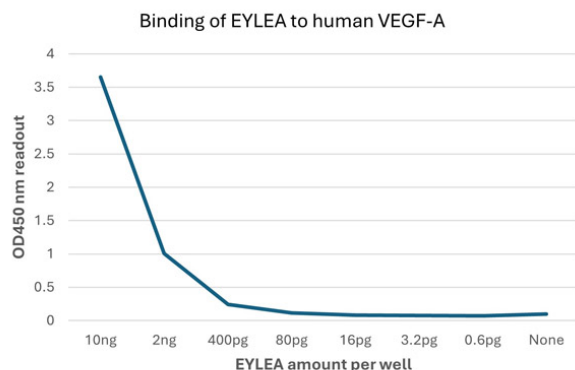
Lanes 3, 5, and 7 (NR): VEGF165 non-reduced.

Protein Load: Lane 2-3: 200ng. Lane 4-5: 40ng. Lane 6-7: 8ng.

Primary: Humanized Recombinant Human VEGF receptor (p/n 400-001-NG6) at concentration 1ug/mL for 2 hours at 4°C.

Secondary: Goat Anti-Human IgG (H&L) Antibody Peroxidase Conjugated (p/n 609-1302) at 1:5,000 dilution was used.

The VEGF165 protein has a calculated MW of 19.2 kDa. As a result of glycosylation, the protein migrates as 24 kDa (monomer) under reducing (R) condition, and 43-50 kDa (homodimer) under non-reducing (NR) condition (SDS-PAGE).



## ELISA

ELISA results shows the sensitivity of Humanized Recombinant Human VEGF receptor tested against purified human VEGF165 protein. Each well was coated in duplicate with 100ng of human VEGF165. The starting dilution of Biosimilar EYLEA protein was 10ng/well with 5-fold dilution. The secondary antibody, Goat Anti-Human IgG (H&L) Antibody Peroxidase Conjugated (p/n 609-1302) with 1:5,000 dilution was used.

## 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.