SibTech, Inc.

[C4]-Monothiol scVEGF
(Deprotected scVEGF)

Product #SBT300

[C4]-Monothiol scVEGF is an engineered 28 kDa single-chain vascular endothelial growth factor with a single C4 thiol group available for site-specific radiolabeling with ^{99m}Tc and conjugation of various payloads. The protein consists of two 3-112aa fragments of human $VEGF_{121}$ cloned head-to-tail and fused to an N-terminal Cys-tag with C4-thiol group. The complete amino acid sequence has the following 242 amino acids:

Met Lys Glu Ser **Cys** Ala Lys Lys Phe Gln Arg Gln His Met Asp Ser Gly Gly Gly Gly Ser Met
Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln Arg Ser Tyr
Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe
Lys Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val
Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln Gly Gln His Ile
Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Ala
Met Ala Glu Gly Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln Arg Ser
Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile
Phe Lys Pro Ser Cys Val Pro Leu Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys
Val Pro Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His Gln Gly Gln His
Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg

Preparation: [C4]-Monothiol scVEGF is produced in *E. Coli* and purified from inclusion bodies with SH-group of C4 residue "protected" in a mixed disulfide with glutathione. To obtain "deprotected" scVEGF, mixed disulfide bond is cleaved under controlled reducing conditions. [C4]-Monothiol scVEGF is then purified using RP-HPLC and lyophilized directly from the RP HPLC eluate (0.1% aqueous TFA / AcCN).

^{99m}Tc radiolabeling and other applications: [C4]-Monothiol ctEGF can be directly radiolabeled with ^{99m}Tc on Cys-tag for SPECT imaging of VEGF receptors, or used for site-specific conjugation of various payloads to Cys-tag (1-3).

One vial contains 0.15 mg of lyophilized [C4]-Monothiol scVEGF

Reconstitution: To insure full recovery, centrifuge the vial briefly before opening. Reconstitute in 0.15 ml of a buffer of your choice, to a final concentration of 1 mg/ml. We do not recommend using less than 0.15 ml for reconstitution. Because the protein is supplied as TFA salt, we recommend using buffers with a sufficient buffer capacity (at least 50 mM).

Stability: Lyophilized [C4]-Monothiol scVEGF is stable for 1 year at -70^{o}C. Once reconstituted at neutral to alkaline pH, C4-thiol groups of deprotected scVEGF will oxidize rapidly to form covalent (S-S-bound) scVEGF dimers. Therefore, to ensure efficient use of C4-thiol group for radiolabeling or conjugation, deprotected scVEGF has to be used immediately after reconstitution.

Safety warnings: For research use only. Not for human use. Not recommended or intended for diagnosis in humans or animals. As all chemicals should be considered as potentially hazardous, it is advisable to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

References

1. Backer MV, Levashova Z, Patel V, Jehning BT, Claffey K, Blankenberg FG, Backer JM. Molecular imaging of VEGF receptors in angiogenic vasculature with single-chain VEGF driven probes. *Nature Med,* 13, 504-509, 2007
2. Backer MV, Levashova Z, Levenson R, Blankenberg FG, Backer JM. Cysteine-containing fusion tag for site-specific conjugation of therapeutic and imaging agents to targeting proteins. Methods in Molecular Medicine. Peptide-based Drug Design. Humana Press, New York, NY. Ed: L. Otvos. Vol. 494, p.275-94, 2008.
3. Levashova Z, Backer MV, Backer JM, Blankenberg FG. Direct labeling of Cys-tag in scVEGF with technetium 99m. *Bioconjugate Chemistry,* 19:1049-54, 2008.

115A Commerce Drive, Brookfield CT 06804, Tel. +1 203 775-5677, FAX +1 203 775-5705
www.sibtech.com E-mail: info@sibtech.com