

SibTech, Inc.

[C4]-Monothiol ctEGF (Deprotected ctEGF)

SBT200

[C4]-Monothiol Cys-tagged (ct) EGF is an engineered 9.3 kDa human epidermal growth factor with a single C4 thiol group available for site-specific radiolabeling with ^{99m}Tc or conjugation of various payloads. The protein consists of human EGF fused to an N-terminal Cys-tag (ct) via a $(\text{G}_4\text{S})_3\text{MG}$ linker. Complete amino acid sequence of ctEGF has the following 86 amino acids:

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

Deprotection: [C4]-Monothiol ctEGF 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" ctEGF, mixed disulfide bond is cleaved under controlled reducing conditions. [C4]-Monothiol ctEGF is then purified using RP-HPLC and lyophilized from 20 mM ammonium acetate at pH 5.0.

Radiolabeling with ^{99m}Tc and other applications: [C4]-Monothiol ctEGF can be directly radiolabeled with ^{99m}Tc on Cys-tag for SPECT imaging of EGF receptors (1), or used for site-specific conjugation of various payloads to Cys-tag (1, 2).

One vial contains 0.1 mg of essentially salt-free lyophilized [C4]-Monothiol ctEGF

Reconstitution: To insure full recovery, centrifuge the vial briefly before opening. Reconstitute in 0.1 ml of buffer of your choice, to a final concentration of 1 mg/ml. We do not recommend using less than 0.1 ml for reconstitution.

Stability: Lyophilized deprotected ctEGF is stable for 1 year at -20°C . Once reconstituted at neutral to alkaline pH, C4-thiol groups of deprotected ctEGF will oxidize rapidly to form covalent (S-S-bound) ctEGF dimers. Therefore, to ensure efficient use of C4-thiol group for radiolabeling or conjugation, deprotected ctEGF 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. Levashova Z, Backer M, Horng G, Felsher D, Backer JM, Blankenberg FG. SPECT and PET Imaging of EGF Receptors with Site-Specifically Labeled EGF and Dimeric EGF. *Bioconjug. Chem.* 20, 742–749, 2009.
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.