Sibtech, Inc.

EGF/Si reagent

#SBT202

The EGF/Si reagent is based on human EGF fused to an N-terminal Cys-tag (1-15aa fragment of human RNase I with the R4C amino acid substitution) via a $(G_4S)_3$ linker. Molecular weight of the resulting protein is 9.3 kDa. Complete amino acid sequence of Cys-tagged EGF is as follows:

Cys-tag	*Met* - Lys Glu Ser Cys Ala Lys Lys Phe Gln Arg Gln His Met Asp Ser
$(G_4S)_3$ linker	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
Di-peptide due to cloning into *Nco*I site	Met Gly
Human EGF ORF	Asn 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

The EGF/Si reagent carries single Cy5.5 dye (FW 1128Da; GE Healthcare) attached site-specifically to C4 residue of Cys-tag. One vial contains 150 μg of RP-HPLC purified lyophilized EGF/Si

Purity: This EGF/Si preparation contains >98% pure protein that migrates as a single band with an apparent molecular weight of ~10,000 daltons in reducing SDS-PAGE. The protein and dye concentrations were calculated using integral absorption in RP-HPLC profiles at 216 nm and 598 nm, respectively.

Functional activity in tissue culture: The ability of EGF/Si to bind to and activate EGF receptor was tested in two tissue culture assays: stimulation of EGFR tyrosine autophosphorylation and competition with EGF-toxin fusion protein (SibTech product # SBT076.012) for binding to EGFR expressed on MDA-231luc cells (SibTech product # 093.MDA. Relative to unmodified human EGF (Sigma), in both assays EGF/Si displayed >95% EGF activity

Stability: EGF/Si is shipped lyophilized. After reconstitution, it should be aliquoted and frozen for long-term storage. Freezing-thawing should be avoided.

Handling:
Good laboratory technique should be employed in the safe handling of this product. This requires observing the following practices:

1. Wear lab coat, gloves and safety glasses
2. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with EGF/Si
3. Avoid accidental autoinjection by exercising extreme care when handling in conjunction with any injection device.
4. EGF/Si is intended for research purposes only. NOT FOR HUMAN USE.
5. SibTech, Inc. is not liable for any damages resulting from the misuse or handling of EGF/Si

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