Abstract: $0$<strong>Introduction:</strong> EGFR overexpression is characteristic of various malignancies and could be considered as an excellent object for designing specific inhibitors such as anti-EGFR monoclonal antibodies (mAb) for cancer therapy. Drawbacks exerted by large size of full-length antibodies leads to development of single chain antibodies that benefit from smaller size and short circulation half-lives. In this paper, we describe the engineering of monoclonal antibody C225 into a single chain form and evaluation of its reactivity with cells expressing high level of EGFR.$0$0$0$<strong>Method:</strong> The RNA extracted from C225 cells was reverse transcribed to cDNA and used for PCR amplification of genes encoding light and heavy chains variable regions. The PCR products were cloned and expressed in <em>E. coli </em>BL21 for production. $0$0$0$The expressed protein was analyzed by SDS-PAGE and purified by Ni-NTA affinity chromatography. Analysis of reactivity of purified C225-scFv with EGFR expressing A431 tumor cell line was tested by western blotting and enzyme-linked immunosorbent assay. $0$0$0$<strong>Results:</strong> The results indicated that C225-scFvs are highly expressed in <em>E. coli </em>and appeared as a 26 kDa protein in SDS-PAGE analysis of induced cells. $0$0$0$<strong>Conclusion:</strong> In conclusion the results of this study indicated that C225-scFv produced in this study is capable to bind to EGFR and according to previous studies it could be used in diagnosis and treatment of EGFR over-expressing tumoral cells.$0$