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# Enhancing the Soluble Expression and Functionality of anti-CD22 Single-Chain Antibody Fragments in *Escherichia coli*

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**ABSTRACT**

**Introduction:** CD22 is a surface antigen predominantly found in cancerous B-cell lineages, making it a significant target for biopharmaceuticals to treat hematologic B-cell malignancies. Biopharmaceuticals, including vaccines, cell and gene therapies, and recombinant protein therapies, have revolutionized medical treatments. Monoclonal antibodies (mAbs)are prominent in this field but face challenges such as stability, aggregation, and post-translational modifications. To address these issues, researchers have developed single-chain variable fragments (scFvs), which are smaller and potentially more stable derivatives of mAbs.

**Methods and Materials:** An anti-CD22-scFv was designed and synthesized for expression *in Escherichia coli*. The protein was purified using immobilized metal affinity chromatography (IMAC) based on column refolding and purification and native purification. We optimized expression conditions, including post-induction temperature, post-induction time, and Isopropyl β-D-1-thiogalactopyranoside (IPTG) concentration, to maximize the soluble protein yield. The binding capacity of the hybrid- and native-purified proteins to $CD22^+$ Raji and CD22-K562 cells was assessed using flow cytometry. The efficacy of anti-CD22-scFv in inhibiting the proliferation of $CD22^+$ Raji cells was evaluated using the MTT assay.

**Results:** The anti-CD22-scFv protein was successfully expressed in the Rosetta (DE3) strain using the $pET\text{-}28a^+$ expression vector but not in the Rosetta-gami 2 strain. Hybrid purification followed by 12% SDS-PAGE and Coomassie brilliant blue staining confirmed the protein expression. Initial solubility tests indicated that the anti-CD22-scFv protein was insoluble. Optimizing the conditions to an IPTG concentration of 0.5 mM, a post-induction temperature of 25 °C, and an eight-hour post-induction time yielded higher solubility. Native-purified and hybrid-purified anti-CD22-scFvs showed binding affinities of 91.4% and 84% to $CD22^+$ Raji cells, respectively. The anti-CD22-scFv significantly reduced the proliferation of $CD22^+$ Raji cells.

**Conclusion and Discussion:** This study demonstrates the effectiveness of a one-step IMAC-based purification method for isolating anti-CD22-scFv protein from recombinant *E. coli*, combining the purification and refolding into a single, cost-effective step. Optimization of the environmental parameters, including expression strain, post-induction temperature, post-induction time, and IPTG concentration, significantly improved the protein yield and solubility. The anti-CD22-scFv shows promising potential in targeting and reducing the proliferation of $CD22^+$ tumor cells, highlighting its therapeutic potential for B-cell malignancies.

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