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Isolation, Purification and Refolding of the Recombinant Bovine α-Interferon From Inclusion Bodies

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Aim of the study: To carry out isolation, purification and refolding by dilution of the recombinant bovine α-interferon (rb α-IFN).

Material and Methods: The following methods were used in the research: UV/Visible spectrophotometry for the determination of the best washing and solubilizing solutions for inclusion bodies (IB), protein concentration and the effectiveness of refolding systems by turbidimetric assay; electrophoretic method for the target protein visualization and assessing its purity; and anion-exchange liquid chromatography for rb α-IFN purification from impurities and collection the protein of interest at the final stage.

Results: Application of two step IB washing by solutions containing 50 mM Tris, 50 mM NaCl and 3,5 M Urea, its further solubilization in 50 mM Tris-HCl, pH 9, 8 M Urea and 10 mM β-mercaptoethanol and subsequent purification on anion-exchange resin makes it possible to get the target protein with the sufficient purity for its renaturation. On the basis of preliminary performed screening of the main characteristics of the refolding buffer, such as pH and temperature value, red-ox potential, final protein and urea concentration in the solution, as well as antiaggregant chemical additives the system containing 10 mM NaPB, pH 7,4, 0,4 M sucrose, 1 mM EDTA, 0,05% cremaphor, 1 mM L-Cys and 0,1 mM L-cystine was chosen as the best one. Scaling up the renaturation process in this refolding system allows to obtain rb α-IFN in active form, homogeneous condition and 20% yield.

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