METHODS TO OBTAIN RECOMBINANT PROTEINS WITH INCREASED SIALYLATION FROM CELLS THAT EXPRESS ADENOVIRUS E1A PROTEIN, AND PROTEINS OBTAINED THEREBY

ABSTRACT

The invention provides compositions comprising one or more isoforms of an erythropoietin (EPO) comprising glycans linked thereto, characterized in that said glycans comprise LewisX structures and on average at least 6 sialic acid moieties per EPO molecule. The invention further provides methods for obtaining a composition comprising one or more isoforms of an erythropoietin (EPO) comprising glycans linked thereto wherein said glycans comprise on average at least 6 sialic acids per EPO molecule and from 0 to 2 Lewis x structures, said method comprising: a) providing a eukaryotic cell containing a nucleic acid sequence encoding an adenoviral E1A protein in expressible format and further containing a nucleic acid encoding an EPO in expressible format, wherein said cell further contains a nucleic acid sequence encoding a sialyltransferase, preferably an alpha-2,6-sialyltransferase or an alpha-2,3-sialyltransferase, under control of a heterologous promoter; b) culturing said cell in a serum-free culture medium and allow expression of an EPO in said cell; c) harvesting the expressed EPO from said cell and/or from the culture medium; and d) purifying and fractionating the EPO to obtain fractions which have an increased average sialic acid content of the N-linked glycans per EPO molecule, to obtain a composition comprising one or more iso forms of an EPO comprising glycans linked thereto wherein said glycans co mprise on average at least 6 sialic acids per EPO molecule and from 0 to 2 Lewis x structures.

No Figure