

Title (en)

PROCESS FOR ACTIVATING HETEROLOGOUS, EUKARYOTIC PROTEINS GENETICALLY ENGINEERED AND PRESENTING DISULPHIDE BRIDGES AFTER THEIR EXPRESSION IN PROCARYOTIC CELLS.

Title (de)

VERFAHREN ZUR AKTIVIERUNG VON GENTECHNOLOGISCH HERGESTELLTEN, HETEROLOGEN, DISULFIDBRÜCKEN AUFWEISENDEN EUKARYONTISCHEN PROTEINEN NACH EXPRESSION IN PROKARYONTEN.

Title (fr)

PROCEDE D'ACTIVATION DE PROTEINES EUKARYOTES HETEROLOGUES PRODUITES PAR GENIE GENETIQUE ET PRESENTANT DES PONTS BISULFURES, APRES LEUR EXPRESSION DANS DES PROCARYOTES.

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Application

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Abstract (en)

[origin: WO8702673A2] To activate heterologous, procaryotic proteins genetically engineered and presenting disulphide bridges after their expression in procaryotic cells, cellular disintegration, solubilisation under denaturing and reducing conditions and activation under oxidating conditions in the presence of GSH/GSSG, either one works during the activation stage with a pH value between 9 and 12, a GSH concentration between 0.1 and 20 mmol/l, a GSSG concentration between 0.01 and 3 mmol/l and a non-denaturing concentration of the denaturing agent, or one separates the reducing and denaturing agents, transforms the thiol groups of the proteins in the mixed disulphides of protein and glutathione by adding GSSG under denaturing conditions and sets during the activation stage a pH value between 7 and 10.5, a GSH concentration between 0.5 and 5 mmol/l and a non-denaturing concentration of the denaturing agent. This process is particularly useful to obtain t-PA and interferon beta .

Abstract (fr)

Pour activer des protéines eucaryotes hétérologues produites par génie génétique et présentant des ponts bisulfures après leur expression dans des procaryotes, la désaggrégation des cellules, leur solubilisation dans des conditions dénaturantes et réductrices et leur activation dans des conditions oxydantes en présence de GSH/GSSG, soit on utilise pendant l'étape d'activation un pH compris entre 9 et 12, une concentration de GSH comprise entre 0,1 et 20 mmol/l, une concentration de GSSG comprise entre 0,01 et 3 mmol/l et une concentration non-dénaturante de l'agent dénaturant, soit on sépare les agents réducteur et dénaturant, transforme les groupes thiols des protéines dans les bisulfures mixtes de protéine et de glutathion par adjonction de GSSG dans des conditions dénaturantes et on utilise pendant l'étape d'activation un pH compris entre 7 et 10,5, une concentration de GSH comprise entre 0,5 et 5 mmol/l et une concentration non-dénaturante de l'agent dénaturant. Ce procédé est particulièrement utile pour obtenir du t-PA et de l'interféron beta.

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