CLAIMS

1. A method for purifying transgenic human Factor VII (TgFVII) and/or transgenic activated human Factor VII (TgFVIIa) ~~from a source biological material~~ produced in the milk of transgenic non-human animal, comprising a step of affinity chromatography comprising the steps of:

(a) contacting ~~a source biological material~~ the milk containing human TgFVII and/or human TgFVIIa with a ligand which is specific to human TgFVII and/or human TgFVIIa, under conditions allowing the human TgFVII and/or human TgFVIIa to bind the ligand , and

(b) recovering human TgFVII and/or human TgFVIIa by disrupting in a non destructive way the interaction with said ligand,

wherein said ligand .

~~2. The method according to claim 1, wherein said ligand~~ is an antigen-binding protein directed to at least one human TgFVII or human TgFVIIa epitope, or a functional fragment or derivative thereof.

~~3~~2. The method according to claim~~s~~ 1 ~~or 2~~, wherein said ligand comprises two heavy polypeptide chains forming two complete antigen binding sites and is devoid of light polypeptide chains.

~~4~~3. The method according to claims 1 to ~~3~~2, wherein said ligand is derived from heavy immunoglobulin chains of Camelidae.

~~5~~4. The method according to claims 1 to ~~4~~3, wherein step (b) comprises at least one washing step for removing unbound material before eluting human TgFVII or human TgFVIIa from the ligand, and further optionally comprises a second washing step for removing weak bound material.

~~6. The method according to claims 1 to 5, wherein the source biological material is obtained from a transgenic animal producing TgFVII and/or TgFVIIa.~~

~~7~~5. The method according to claim ~~6~~1, wherein the animal is a mammal female, and preferably a rabbit female~~, and the source biological material is milk~~.

~~86~~. The method according to claim ~~57~~, further comprising the preliminary steps, before step (a), of collecting and clarifying milk.

~~97~~. The method according to claim ~~86~~, wherein the milk clarification step is performed by citrate salt addition, followed by filtration.

~~108~~. The method according to claims 1 to ~~97~~, further comprising a step c) of chromatographic purification of the eluted human TgFVII and /or human TgFVIIa on an ion exchanger, and preferably on an anion exchanger, under conditions wherein human TgFVII and/or human TgFVIIa are retained on the ion exchanger.

~~119~~. The method according to claims 1 to ~~108~~, further comprising a step d) of chromatographic separation of the eluted human TgFVII and/or human TgFVIIa on pseudo-affinity resin, and preferably on hydroxyapatite, under conditions wherein human TgFVII and/or human TgFVIIa are retained on the pseudo-affinity resin.

~~1210~~. The method according to claims 1 to ~~119~~, further comprising a step e) of chromatographic separation of the eluted human TgFVII and/or human TgFVIIa on a size exclusion support.

~~1311~~. The method according to claims 1 to ~~1210~~, further comprising at least one step of eliminating and/or inactivating viruses, preferably by nanofiltration and/or by Solvent/Detergent treatment.

~~1412~~. The method according to claims 1 to ~~1311~~, further comprising the final steps of formulating, sterilizing and lyophilizing the purified human TgFVII and/or human TgFVIIa.

~~1513~~. The method according to claims 1 to ~~1412~~, wherein the step of affinity chromatography comprises the steps of:

(a) loading a chromatographic resin on which a ligand as defined in any of claims 1 to 4 is immobilized, with milk from a transgenic female rabbit containing recombinantly produced human TgFVII and/or human TgFVIIa, under conditions allowing the ligand to bind human TgFVII and/or human TgFVIIa;

(b1) washing the unbound material;

(b2) washing the weak bound material; and

(b3) eluting human TgFVII and/or human TgFVIIa from the resin.

146. The method according to claims ~~5~~ 4 to ~~15~~13, wherein, during said affinity chromatography step, said second washing step for removing weak bound material is performed with a buffer comprising from 10% to 50% of a hydrophobic agent, and the ionic strength of which is ranging from 200 to 600 mM.

~~17~~15. The method according to claims ~~5~~ 4 to ~~16~~14, wherein, during said affinity chromatography step, said elution step for eluting human TgFVII and/or human TgFVIIa from the resin is performed with a buffer comprising from 20% to 70% of a hydrophobic agent, and the ionic strength of which is ranging from 500 to 2500 mM.

~~18~~16. The method according to claims 1 to ~~17~~15, wherein the resulting purified transgenic FVII is an almost fully activated, high purity human FVII, comprising a low percentage of degraded, proteolized or oxidized forms.

~~19~~17. The method according to claims 1 to ~~18~~16, characterized in that the affinity chromatography step and/or of ion exchange chromatography step and/or of size exclusion chromatography step allow to obtain a viral reduction factor superior to 3 log_{10}, preferably superior to 4 log_{10}, more preferably superior to 5 log_{10}.