

CLAIMS

1. A process for producing a membrane protein comprising the steps of:

- 5 a. Expressing a membrane protein in a host organism present in an aqueous medium,
- b. Liberating the membrane protein from the host organism,
- c. Adding a detergent solution to solubilize the membrane protein,
- 10 d. Recovering a liquid fraction of the solubilized membrane protein as the supernatant by centrifugation,
- e. Subjecting the liquid fraction to chromatography to bind or retain the membrane protein on a stationary phase, and
- 15 f. Eluting the stationary phase with an elution buffer to produce the membrane protein

wherein the centrifugation in step d is performed at 500 g to 30,000 g.

2. The process according to claim 1, wherein the aqueous medium comprising the host organism of step a is filtered prior to the liberating of the host organism according to step b.

3. The process according to claim 2, wherein the aqueous medium comprising the host organism of step a is filtered through a microfiltration membrane having a pore diameter of 0.5 micrometer or less.

4. The process according to anyone of the claims 1 to 3, wherein the host organism is isolated, optionally after the filtration, by centrifugation of the aqueous medium comprising the host organism.

5. The process according to anyone of the claims 1 to 4, wherein the centrifugation is performed at 500 g to 30.000 g, preferably at 1,000 g to 10,000 g.

6. The process according to anyone of the claims 1 to 5,
5 wherein the isolated host organism is washed with an isotonic saline solution to dissolve contaminating salts and subsequently centrifuged to isolate the washed host organism.

7. The process according to anyone of the claims 1 to 6, wherein a dilution buffer is added prior to step b.

10 8. The process according to anyone of the claims 1 to 7, wherein an aqueous lysis solution is added to liberate the membrane protein from the host organism.

9. The process according to anyone of the claims 1 to 8, wherein the aqueous lysis buffer is a detergent solution,
15 which simultaneously solubilizes the membrane protein.

10. The process according to anyone of the claims 1 to 9, wherein the host cells are allowed to be subjected to the action of the detergent during agitation.

11. The process according to anyone of the claims 1 to 7,
20 wherein the liberation of the membrane protein from the host cells is performed by a homogenizer.

12. The process according to anyone of the claims 1 to 11, wherein a cationic flocculant is added to the liberated membrane protein to form a suspension.

25 13. The process according to anyone of the claims 1 to 12, wherein the cationic flocculant is a polyamine compound, such as Superfloc C581.

14. The process according to anyone of the claims 1 to 13, wherein the cationic flocculant is allowed to react with the
30 cell parts of the resuspension during gentle agitation to form flocs.

15. The process according to anyone of the claims 1 to 14, wherein the liquid fraction of step d is recovered as the supernatant from a centrifugation of the suspension containing the flocs.

5 16. The process according to anyone of the claims 1 to 7, wherein the liquid fraction of step d is recovered from a resuspension of a solid fraction, wherein the solid fraction is resuspended in a detergent solution for solubilizing the membrane protein.

10 17. The process according to claim 16, wherein the liquid fraction of step d is harvested as the supernatant by centrifugation.

18. The process according to anyone of the claims 1 to 17, wherein the detergent is selected from the group consisting
15 of lauryl dimethylamine N-oxide (LDAO), octyl glucoside (OG), dodecyl maltoside (DDM) or combinations thereof.

19. The process according to anyone of the claims 1 to 18, wherein the centrifugation is performed at 500 g to 30.000 g, preferably at 1,000g to 10,000g.

20 20. The process according to anyone of the claims 1 to 19, wherein the stationary phase capable of binding or retaining the membrane protein is present in a column.

21. The process according to anyone of the claims 1 to 20, wherein the membrane protein is associated with a first part
25 of an affinity pair and the stationary phase is associated with a second part of the affinity pair.

22. The process according to anyone of the claims 1 to 21, wherein the first part of the affinity pair is a histidine tag.

23. The process according to anyone of the claims 1 to 22,
wherein the histidine tag comprises 8 or more histidine
molecules.

24. The process according to anyone of the claims 1 to 23,
5 wherein the eluting buffer comprises imidazole.

25. The process according to anyone of the claims 1 to 24,
wherein the column prior to the elution with the elution
buffer, is washed with a washing buffer comprising 40% or
less of the concentration of imidazole in the elution buffer.

10 26. The process according to anyone of the claims 1 to 25,
wherein the concentration of imidazole in the elution buffer
is 400mM or more.