

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.

20 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 through a microfiltration membrane having a pore diameter of 0.5 micrometer or less.

25 3. The process according to claim 1 or 2, wherein the host organism is isolated, optionally after the filtration, by centrifugation of the aqueous medium comprising the host organism, said centrifugation being performed

at 500 g to 30.000 g.

4. The process according to anyone of the claims 1 to 3,
wherein the isolated host organism is washed with an
isotonic saline solution to dissolve contaminating salts and
5 subsequently centrifuged to isolate the washed host
organism.

5. The process according to anyone of the claims 1 to 4,
wherein a dilution buffer is added prior to step b.

6. The process according to anyone of the claims 1 to 5,
10 wherein an aqueous lysis solution is added to liberate the
membrane protein from the host organism.

7. The process according to claim 6, wherein the aqueous
lysis buffer is a detergent solution, which simultaneously
solubilizes the membrane protein.

15 8. The process according to anyone of the claims 1 to 5,
wherein the liberation of the membrane protein from the host
cells is performed by a homogenizer.

9. The process according to anyone of the claims 1 to 8,
wherein a cationic flocculant is added to the liberated
20 membrane protein to form a suspension.

10. The process according to claim 9, wherein the cationic
flocculant is a polyamine compound.

11. The process according to claim 9 or 10, wherein the
cationic flocculant is allowed to react

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with the cell parts of the resuspension during gentle agitation to form flocs, wherein the liquid fraction of step d is recovered as the supernatant from a centrifugation of the suspension containing the flocs.

5 12. The process according to claim 1, 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 13. The process according to claim 12, wherein the liquid fraction of step d is harvested as the supernatant by centrifugation.

14. The process according to anyone of the claims 7 to 13, wherein the detergent is selected from the group consisting of lauryl dimethylamine N-oxide (LDAO), octyl glucoside (OG), dodecyl maltoside (DDM) or combinations thereof.

15 15. The process according to anyone of the claims 1 to 14, wherein the centrifugation of step d is performed at 1,000g to 10,000g.

16. The process according to anyone of the claims 1 to 15, 20 wherein the membrane protein is associated with a first part of an affinity pair and the stationary phase is associated with a second part of the affinity pair.

17. The process according to claim 16, wherein the first part of the affinity pair is a histidine tag comprising 8 or more histidine molecules.

18. The process according to anyone of the claims 1 to 17,
5 wherein the eluting buffer comprises imidazole.

19. The process according to claim 18, wherein the stationary phase capable of binding or retaining the membrane protein is present in a column and the column prior to the elution with the elution buffer, is washed with a
10 washing buffer comprising 40% or less of the concentration of imidazole in the elution buffer.

20. The process according to claim 18 or 19, wherein the concentration of imidazole in the elution buffer is 400mM or more.