GE Healthcare

Membrane Protein Purification Kit

Membrane Protein Purification Kit (Fig 1) is designed for efficient detergent screening of histidine-tagged membrane proteins. Small aliquots of cell membranes are solubilized in different detergents followed by rapid purification using His Mag Sepharose™ Ni. The purification step is performed directly after solubilization using the same detergent. Analysis and evaluation can be performed by a number of methods such as Western blot, gel filtration, or light scattering.

Key benefits:

- Easy solubilization and purification of membrane proteins
- Rapid detergent screening in the convenient magnetic bead format
- Simultaneous evaluation of solubilization and purification efficiency
- Ready-to-use detergent solutions
- Easy-to-follow protocol

Membrane Protein Purification Kit contains seven detergents (purity ≥ 99% by HPLC analysis), His Mag Sepharose Ni, buffer stock solutions, and a protocol (Table 1). The detergents are supplied in aqueous stock solutions, eliminating the handling of dry powder detergents. Membrane Protein Purification Kit is sufficient for two complete screening experiments according to the included protocol.

His Mag Sepharose Ni are magnetic beads designed for rapid small-scale purification and screening of histidine-tagged proteins from different sources. The high density of the beads allows rapid capture by magnetic devices, while the visibility of the beads ensures reliable collection of the bound histidine-tagged membrane proteins in the screening and purification procedure. Purification of target membrane proteins for a number of analytical and/or other down-stream applications can be achieved within a few hours. The protocol workflow is outlined in Figure 2.

Fig 1. Membrane Protein Purification Kit allows convenient detergent screening for solubilization and purification of histidine-tagged membrane proteins.

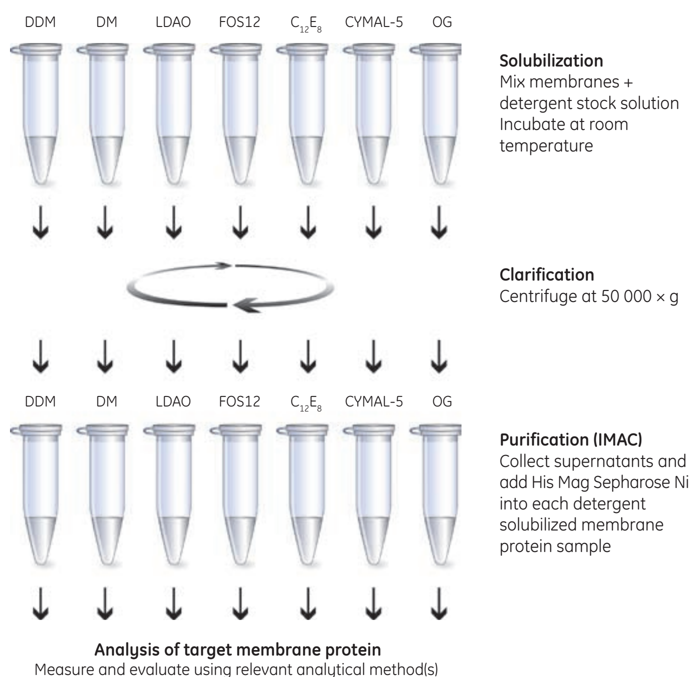


Fig 2. Schematic workflow for detergent screening.

Table 1. Membrane Protein Purification Kit contents*

Component	Amounts supplied	CMC[†] (%)	CMC[†] (mM)	Detergent class
His Mag Sepharose Ni	3 × 1 ml			
n-Dodecyl-β-D-maltoside (DDM)	1 ml[‡]	0.009	0.17	Non-ionic
n-Decyl-β-D-maltoside (DM)	1 ml[‡]	0.09	1.8	Non-ionic
Lauryldimethylamine-N-oxide (LDAO)	1 ml[‡]	0.02	1–2	Zwitter-ionic
n-Dodecylphosphocholine (FOS12)	1 ml[‡]	0.05	1.5	Non-ionic
Dodecyl octaethyleneglycol ether ($C_{12}E_8$)	1 ml[‡]	0.005	0.09	Non-ionic
Cyclohexyl-1-pentyl-β-D-maltoside (CYMAL™-5)	1 ml[‡]	0.12	2–5	Zwitter-ionic
n-Octyl-β-D-glucoside (OG)	1 ml[§]	0.53	18–20	Non-ionic
Phosphate buffer (stock) pH 7.4 (160 mM sodium phosphate, 4 M NaCl)	100 ml			
2 M imidazole pH 7.4	100 ml			

* All detergents are from Anatrace™ Maumee, Ohio, USA (Affymetrix).
[†] Approximate critical micelle concentrations (CMC) in water at 20°C.
[‡] 10% (w/v) aqueous solution.
[§] 20% (w/v) aqueous solution.

Solubilization and purification of histidine-tagged membrane proteins

Integral membrane proteins play major roles in fundamental biological processes, such as transport of molecules, signaling, metabolism, and maintaining cell and tissue structures. Today, they are main targets for the development of new pharmaceuticals.

Integral membrane proteins transverse the cell membrane and are embedded in and associated with the lipids of the cell membrane environment. To be able to study these proteins, they must be dispersed in an aqueous solution. This is accomplished by adding a detergent that solubilizes the membrane and forms soluble complexes with lipids and proteins.

To avoid protein loss and inactivation during solubilization and concomitant purification of integral membrane proteins, the choice of detergent is a key factor. Some important features of detergents include critical micelle concentration, ionic/non-ionic character, and chain length. These features must be considered in close context to the aim of the solubilization/purification of the target protein. One detergent might be useful for solubilization/purification whereas another is more suitable for crystallization experiments. This also depends on the characteristics of the target protein. Therefore, a detergent screen is often necessary to find the optimal detergent for each purpose.

To be able to study integral membrane proteins, they are often recombinantly expressed, frequently with an affinity tag. In particular, the histidine-tag is widely used. Membrane Protein Purification Kit facilitates detergent screening, allowing solubilization and purification efficiency to be evaluated simultaneously.

Applications

Screen for optimal detergent(s) for solubilization and purification of histidine-tagged cytochrome bo$_3$ ubiquinol oxidase

Membrane Protein Purification Kit was used to find suitable detergent(s) for solubilization and purification of histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase expressed in *E. coli*. Solubilization and concomitant IMAC purification of this membrane protein was performed using the seven different detergents in the kit.

Analysis was done by SDS-PAGE/Western blot. For detection, enhanced chemoluminescence using ECL™ Plus Western blot detection system was employed. Band intensities were measured for quantitative evaluation.

A size-distribution analysis was also performed by gel filtration chromatography using a Superdex™ 200 5/150 GL column in the presence of the same detergent used for each solubilization and purification experiment.

Detergent concentrations for solubilization and purification are given in Table 2.

Table 2. Detergents and concentrations used in screening

Detergents	Solubilization	Purification
DDM	1%	0.1%
DM	1%	0.2%
LDAO	1%	0.2%
FOS12	1%	0.1%
$C_{12}E_8$	1%	0.1%
CYMAL-5	1%	0.2%
OG	2%	1%

No histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase material could be seen in the flowthrough fractions. This was found for all seven detergents and indicates that all histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase material applied was bound to the His Mag Sepharose Ni beads (data not shown).

The amount of eluted histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase varied between the different detergents with DDM and DM showing the highest amounts (Fig 3). $C_{12}E_8$ showed lower amount/yields.

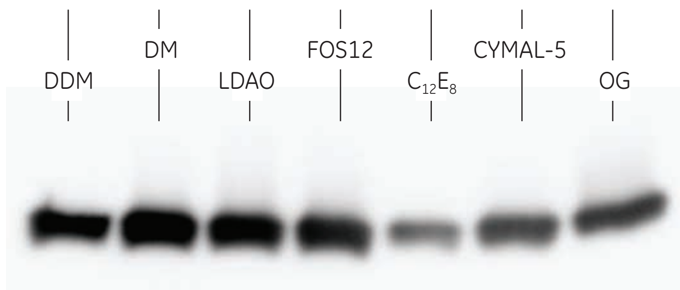


Fig 3. Western blot analysis of the eluted fractions obtained in a detergent screen for purification of total histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase.

A closer quantitative comparison (Fig 4) showed that DDM, DM, and LDAO gave high yields of purified target protein. CYMAL-5 and OG showed somewhat lower values, and the lowest value was found for $C_{12}E_8$; this value was approx. 30% of the value for DM.

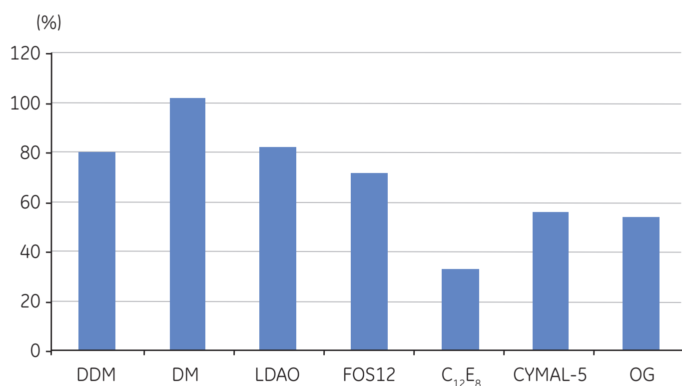


Fig 4. Western blot analysis: Quantitative determination of amount of solubilized and IMAC-purified histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase in different detergents. Relative yields, the highest set to 100%.

The size distribution analysis showed high purity and homogeneous size distributions when using FOS12, and less purity but a somewhat higher yield when using LDAO (Fig 5).

In conclusion, this detergent screen shows that DDM, DM, or LDAO are suitable for solubilization and purification of histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase. FOS12 might be considered as well, since this detergent seems to give slightly higher purity and a more homogeneous size distribution.

Running conditions

Column:	Superdex 200 5/150 GL
Sample load:	25 µl
Buffer:	10 mM phosphate buffer, 150 mM NaCl including the same detergent used for solubilization and purification
Flow rate:	0.3 ml/min
System:	ÄKTA™ design

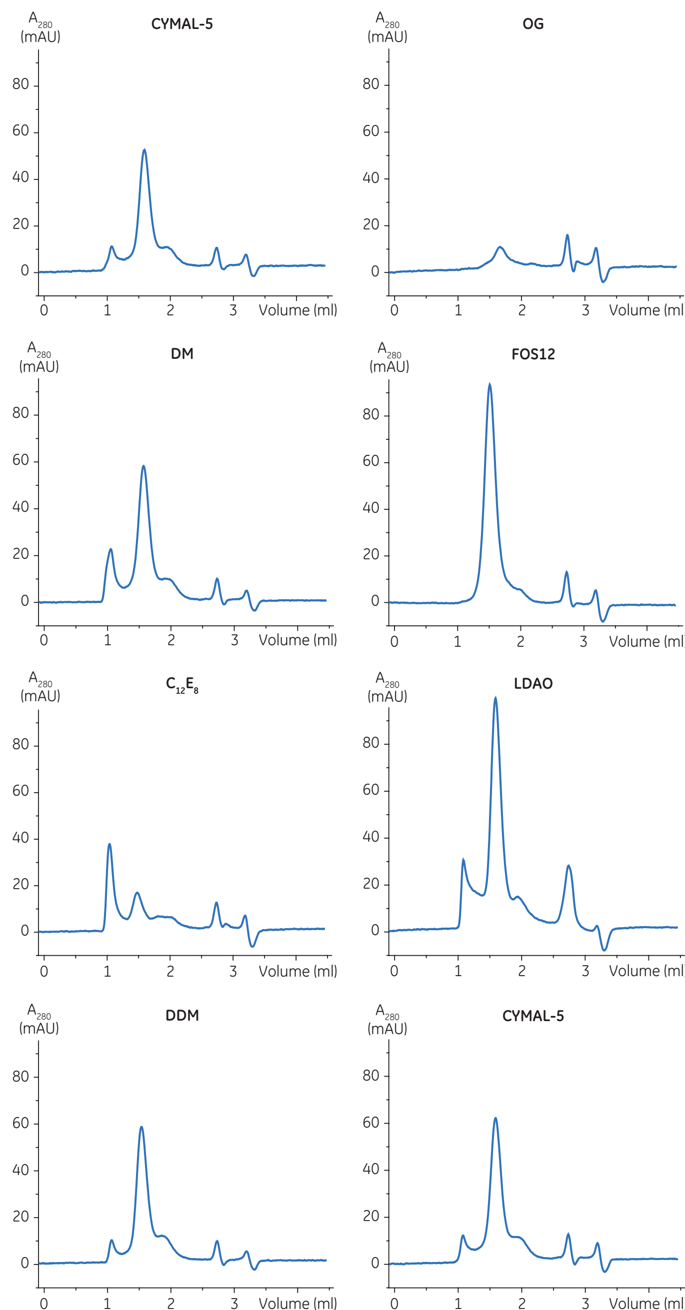


Fig 5. Size-distribution analysis by gel filtration of solubilized and purified histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase. Analysis was performed in the same detergent (denoted in each chromatogram) as for the respective IMAC-purification. CYMAL-5 prepared histidine-tagged cytochrome *bo*$_3$ ubiquinol oxidase was run first and last to ensure stable column performance.

Scale-up purification of YedZGFP-His

Preparation of higher quantities of target membrane protein might be required. After screening for suitable detergent using Membrane Protein Purification Kit, it is straightforward to scale-up the purification to HisTrap™ HP 1 ml or 5 ml columns.

A detergent screen for YedZGFP-His expressed in *E. coli*, analyzed by SDS-PAGE, and quantified by fluorescence detection showed that DM and CYMAL-5 (Fig 6) were suitable detergents for solubilization/purification of YedZGFP-His (detergent concentrations for screening are given in Table 2).

In the scale-up experiment, DM was chosen as detergent. Purification was performed on a HisTrap HP 1 ml column. The YedZGFP-His containing sample was first solubilized in 1% DM. After clarification, a sample volume of 25 ml was loaded in the presence of 0.2% DM and eluted in one step by addition of 500 mM imidazole. Analysis and purity evaluation was done by SDS-PAGE and Deep Purple™ staining (Fig 7A).

The target membrane protein turned out to be the main component in the eluate, although the SDS-PAGE profile (Fig 7B) suggests that a second purification step might be required. Similar purity (data not shown) was obtained for the Mag Sepharose Ni beads in the screening experiment.

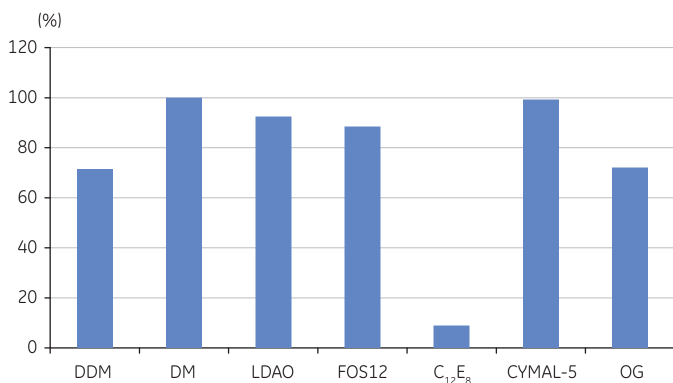
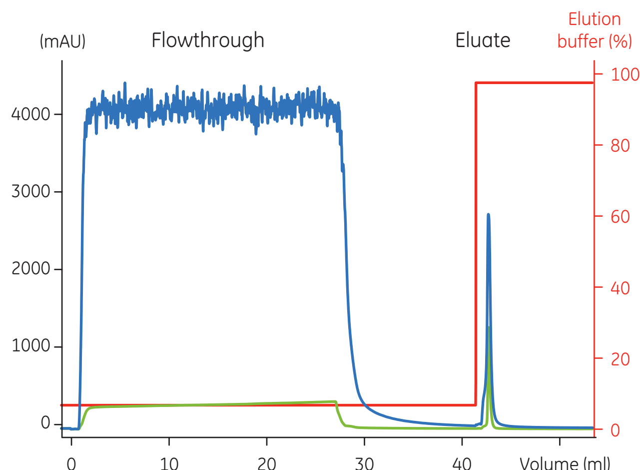


Fig 6. Quantitative determination of the amount of solubilized and His Mag Sepharose Ni-purified histidine-tagged cytochrome bo_3 ubiquinol oxidase in different detergents using SDS-PAGE detection by fluorescence. Relative yield, the highest set to 100%.

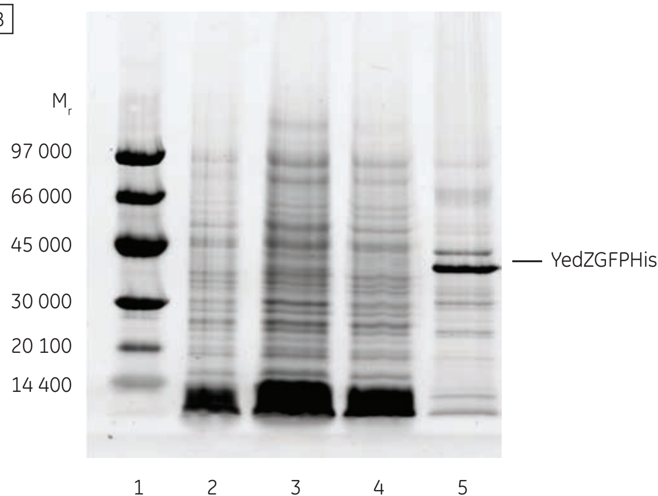
A

Running conditions

Column: HisTrap HP, 1 ml
Sample: YedZGFP-His solubilized in 1% DM for 30 min at 4°C
Sample load: 25 ml
Binding buffer: 20 mM phosphate, 500 mM NaCl, 40 mM imidazole, 0.2% DM, pH 7.4
Elution buffer: 20 mM phosphate, 500 mM NaCl, 500 mM imidazole, 0.2% DM, pH 7.4
Flow rate: 1 ml/min
Detection: A_{490} and A_{280}



B



Lane 1 LMW-SDS Marker Kit
2 Solubilized start material diluted ten-fold
3 Flowthrough fraction diluted five-fold
4 Wash fraction using binding buffer
5 Elution fraction containing YedZGFP-His

Fig 7. Scale-up experiment for purification of YedZGFP-His expressed in *E. coli*. **(A)** Chromatogram from purification on HisTrap HP 1 ml. Flowthrough fraction; Eluate: Elution fraction containing YedZGFP-His. Blue curve (A_{280}), green curve (A_{490}) **(B)** Deep Purple stained SDS-PAGE.

Acknowledgements

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YedZGFP-His material was kindly provided by Dr. Jan-Willem de Gier, Dept. of Biochemistry and Biophysics, Stockholm University, Sweden.

Ordering information

Product	Quantity	Code no.
Membrane Protein Purification Kit	1	28-9805-82

Related products

MagRack 6	1	28-9489-64
His Mag Sepharose Ni	2 × 1 ml	28-9673-88
His Mag Sepharose Ni	5 × 1 ml	28-9673-90
His Mag Sepharose Ni	10 × 1 ml	28-9799-17
HisTrap HP 1ml	5 x 1 ml	17-5247-01
HisTrap HP 5 ml	1 x 5 ml	17-5248-01
HisTrap HP 5 ml	5 x 5 ml	17-5248-02
Ni Sepharose High Performance	25 ml	17-5268-01
Ni Sepharose High Performance	100 ml	17-5268-02
LMW Marker Kit	10 vials	17-0446-01
Full-Range Rainbow™ Molecular Weight Markers	250 µl	RPN800E
Anti-His Antibody	170 µl	27-4710-01
Hybond™-LFP	1 roll	RPN303LFP
ECL Plus Western Blotting Detection system		RPN2132
Hyperfilm™ ECL	1	28-9068-36
Superdex 200 5/150 GL	1	28-9065-61
Superdex 75 5/150 GL	1	28-9205-04
His Buffer Kit	1	11-0034-00

Related literature

Purifying challenging proteins, Principles and Methods	1	28-9095-31
Recombinant Protein Purification Handbook, Principles and Methods	1	18-1142-75

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IMAC Sepharose products and Ni Sepharose products (Histidine-tagged protein purification, Lab products): Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent numbers 5,284,933 and 5,310,663, and equivalent patents and patent applications in other countries (assignee: Hoffman La Roche, Inc

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