GE Healthcare

Instructions 28-9810-90 AA **Challenging proteins**

Membrane Protein Purification Kit

Overview

Membrane Protein Purification Kit is a ready to use kit, that includes His Mag Sepharose™ Ni chromatographic medium, buffers and seven detergents. This convenient kit is designed for efficient screening to find a suitable detergent(s) for solubilization and purification of histidine-tagged membrane proteins.

Membrane Proteins

Membrane proteins are commonly recombinantly expressed and also often histidine-tagged. To be able to handle and study membrane 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 the lipids and membrane proteins. The choice of detergent is the key factor for successful solubilization and further purification of membrane proteins to avoid protein loss, aggregation or inactivation.

Detergent Screening Procedure

To find a suitable detergent for solubilization and purification of histidine tagged membrane proteins, small aliquots of membranes are solubilized in seven different detergents and directly purified on His Mag Sepharose Ni in the presence of each detergent. The purified target membrane proteins can be analyzed further by gel-filtration, western blotting, light scattering or other techniques.

Sepharose

Package contents

Kit content	No. supplied
His Mag Sepharose Ni	3 × 1 ml vial
n-Dodecyl-ß-D-maltoside (DDM)*	10% (w/v) aqueous solution 1 ml
n-Decyl-ß-D-maltoside (DM)*	10% (w/v) aqueous solution 1 ml
Lauryldimethylamine-N-oxide (LDAO)*	10% (w/v) aqueous solution 1 ml
n-Dodecylphosphocholine (FOS12)*	10% (w/v) aqueous solution 1 ml
Dodecyl octaethylene glycol ether ($C_{12}E_8$)*	10% (w/v) aqueous solution 1 ml
5-Cyclohexyl-1-pentyl-ß-D-maltoside (Cymal™ 5)*	10% (w/v) aqueous solution 1 ml
n-Octyl-ß-D-glucoside (OG)*	20% (w/v) aqueous solution 1 ml
Phosphate buffer (stock), pH 7.4 (160 mM sodium phosphate, 4M NaCl)	100 ml
2 M imidazole pH 7.4	100 ml

* All detergents are from Anatrace™ Maumee, Ohio USA (Affymetrix™).

Note: *For easy handling of the magnetic beads you also need MagRack 6, not supplied in the kit, see Order Information.*

Intended use

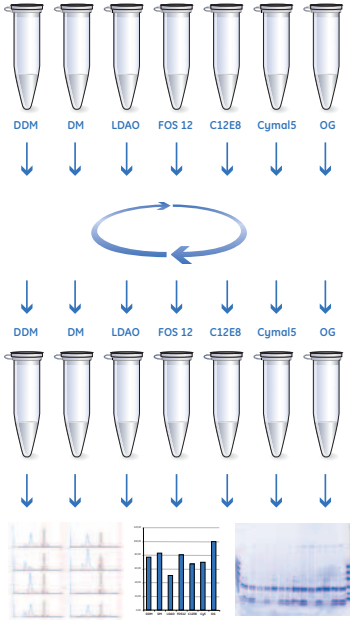
This product is intended for research only, and should not be used in any clinical or *in vitro* procedures for diagnostic purposes.

CAUTION: The product contains nickel, Ni^{2+}, which is potentially allergenic. Always use normal protection equipment like gloves and safety glasses when handling His Mag Sepharose Ni.

Content

1 Description

Detergent screening workflow



Solubilization
Mix membranes + detergent stock solution
Incubate at room temperature

Clarification
Centrifuge at 50 000 × g

Purification (IMAC)
Collect supernatants and add His Mag Sepharose Ni into each detergent solubilized membrane protein sample

Analysis of target membrane protein
Measure and evaluate by use of relevant analytical method(s)

2 General considerations

Characteristics of detergents and buffers

Buffers

Phosphate buffer (stock)[1], pH 7.4	160 mM sodium phosphate, 4 M NaCl
Imidazole pH 7.4	2 M

[1] Formation of salt crystals may occur in the stock solution at low temperatures. Let the stock solution adjust to room temperature before use.

Detergents

Detergent	CMC[1] (%) in water	CMC[1] (mM) in water
n-Dodecyl-ß-D-maltoside (DDM)	0.009	0.17
n-Decyl-ß-D-maltoside (DM)	0.09	1.8
Lauryldimethylamine-N-oxide (LDAO)	0.02	1-2
n-Dodecylphosphocholine (FOS12)	0.05	1.5
Dodecyl octaethylene glycol ether ($C_{12}E_8$)	0.005	0.09
5-Cyclohexyl-1-pentyl-ß-D-maltoside (Cymal 5)	0.12	2-5
n-Octyl-ß-D-glucoside (OG)	0.53	18-20

[1] Critical micellar concentration in distilled water at 20°C

Characteristics of His Mag Sepharose Ni

His Mag Sepharose Ni is an affinity chromatography medium with high affinity for histidine-tagged proteins. The medium consists of magnetic beads based on Sepharose coupled with chelating ligand and charged with nickel ions

Table 1. Characteristics of His Mag Sepharose Ni.

Matrix	Highly crosslinked spherical agarose (Sepharose) including magnetite
Metal ion capacity	~21 µmol Ni^{2+}/ml medium
Binding capacity	Approx. 50 mg water soluble histidine-tagged protein/ml sedimented medium. The capacity was determined using 5 mM imidazole in sample and binding buffer. Note that binding capacity is sample dependent.
Bead size	37 to 100 µm
Working temperature	Room temperature and +4°C
Storage solution	20% ethanol, 5% medium slurry

Note: *His Mag Sepharose Ni is intended for single use only.*

3 Operation

Buffer preparation

The choice of optimal imidazole concentration in the binding buffer is a balance between purity and yield. The recommended imidazole concentrations are 20 mM in the binding buffer and 500 mM in the elution buffer. The tables below specify volumes of supplied phosphate buffer, imidazole solution and detergent stocks required to prepare the binding buffer and the elution buffer. The water and chemicals used for buffer preparation should be of high purity.

Binding buffers

To obtain binding buffers of 20 mM phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4, containing the respective detergents, prepare according to the table below. Final volume will be 4.0 ml.

Note: *For different concentrations of imidazole in the binding buffer please refer to the table in Appendix.*

Binding buffer	Phosphate buffer (stock)	2M imidazole	Distilled water	Detergent stock
0.1% DDM	500 µl	40 µl	3.42 ml	40 µl
0.2% DM	500 µl	40 µl	3.38 ml	80 µl
0.2% LDAO	500 µl	40 µl	3.38 ml	80 µl
0.1% FOS12	500 µl	40 µl	3.42 ml	40 µl
0.1% $C_{12}E_8$	500 µl	40 µl	3.42 ml	40 µl
0.2% Cymal 5	500 µl	40 µl	3.38 ml	80 µl
1% OG	500 µl	40 µl	3.26 ml	200 µl

Elution buffers

To obtain elution buffers of 20 mM phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4, containing the respective detergents, prepare according to the table below. Final volume will be 1.0 ml.

Elution buffer	Phosphate buffer (stock)	2M imidazole	Distilled water	Detergent stock
0.1% DDM	125 µl	250 µl	615 µl	10 µl
0.2% DM	125 µl	250 µl	605 µl	20 µl
0.2% LDAO	125 µl	250 µl	605 µl	20 µl
0.1% FOS12	125 µl	250 µl	615 µl	10 µl
0.1% $C_{12}E_8$	125 µl	250 µl	615 µl	10 µl
0.2% Cymal-5	125 µl	250 µl	605 µl	20 µl
1% OG	125 µl	250 µl	575 µl	50 µl

Suspension buffer (without detergent)

To obtain a buffer of 20 mM phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4:

1.25 ml Phosphate buffer (stock)

100 µl of 2 M imidazole

Add distilled water to final volume 10 ml.

Sample preparation

- Disrupt cells by sonication or other means.
- Ultracentrifuge at 100 000 × g for approx. 1 hour, to remove cell debris and collect membrane pellet.
- Resuspend the membrane pellet in Suspension buffer (see above) to an approximate total membrane protein concentration of 10 mg/ml. This is now the membrane suspension.

Note: *If membranes are resuspended in a different buffer than Suspension buffer, adjust the sample (membrane suspension) to the same pH, salt and imidazole concentration as your binding buffer.*

4 Protocol for detergent screening

Solubilization

1. Prepare seven tubes (1.5 ml), each with 900 µl of membrane suspension, and add 100 µl of the respective 10% (or 20%) detergent stock.
2. Mix by "end-over-end" mixing for 30 min at room temperature.

   Note: *Work in cold room if necessary to preserve protein stability.*
3. Clarify by centrifugation 50 000 × g for approximately 30 min. Collect the supernatants.

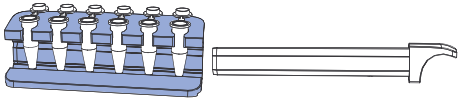
   Note: *If a solubilization analysis will be performed, collect aliquots for Western blot analysis both before, and after clarification - see "Tips and hints", on page 11 for more details.*

Purification

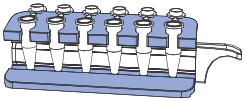
Handling of His Mag Sepharose Ni

It is recommended to use 1.5 ml microcentrifuge tubes and MagRack 6.

- Remove the magnet before adding liquid.



- Resuspend the beads by manually inverting the tubes after adding the liquid.
- Insert the magnet before removing liquid. Make sure to capture all beads to the magnet by manually inverting MagRack 6.



- Use a pipette to remove liquid from the lid, if required.

Magnetic bead preparation

1 Mix the medium slurry thoroughly by vortexing. Dispense 200 µl of suspended magnetic beads into each of the seven microcentrifuge tubes (1.5 ml).

Note: *Mix the medium slurry between each dispensation.*

2 Place the microcentrifuge tubes in MagRack 6.

3 Remove the storage solution.

Equilibration

4 Add 500 µl binding buffer (containing the respective detergent) into each of the seven tubes.

5 Resuspend the beads.

6 Remove the liquid.

Sample application

7 Add 1000 µl of sample. If the sample volume is less than 1000 µl, dilute to 1000 µl with the respective binding buffer.

8 Resuspend the beads and incubate for 30 minutes with slow "end-over-end" mixing.

9 Remove the liquid.

Washing

10 Add 500 µl binding buffer (containing the respective detergent). Resuspend the beads.

11 Remove the liquid.

12 Perform steps 10-11 three times.

Elution

13 Add 100 µl of elution buffer (containing the respective detergent). Resuspend the beads.

14 Remove and collect the eluted material. The eluate should contain most of the purified protein.

15 If desired, repeat the elution.

5 Tips and hints

Buffer preparation

Depending on the stability of the histidine-tagged membrane protein, a lower salt concentration might be an option.

For example, prepare 10 mM phosphate, 250 mM NaCl, by reducing the volume of Phosphate buffer (stock) by half, in both binding and elution buffers, see Tables in "Buffer preparation", on page 7 and 8. Remember to add distilled water to the final volumes.

Solubilization analysis

The supplied protocol includes solubilization of membrane proteins directly followed by IMAC purification in each detergent. This makes it easy to further analyze the purified protein's stability in the seven detergents, before scaling up the purification.

To determine the best detergent for solubilization only, a solubilization analysis can be performed. Collect 10 µl aliquots after solubilization to obtain the total protein fraction and collect 10 µl aliquots of supernatants after clarification to obtain the soluble membrane protein fraction. Quantify by using Western blot analysis or other methods.

Optimization of parameters (His Mag Sepharose Ni)

The purification protocol is suitable for purification of most histidine tagged membrane proteins. However, some parameters for purification may require optimization to obtain the best result. Examples of parameters that may require optimization are amount of beads, incubation time, number of washes and imidazole concentration in sample and binding buffer. See "Appendix", on page 15 for binding buffer recipes with different imidazole concentrations.

6 Troubleshooting

Problem	Possible cause/corrective action
No target membrane protein in the elution fractions	**Expression of target membrane protein is low:** Use higher concentration of total membrane proteins than 10 mg/ml (before solubilization). **Concentration of imidazole in the sample and/ or binding buffer is too high:** The protein is found in the flow-through material. Decrease the imidazole concentration. **Buffer/sample composition is incorrect:** The protein is found in the flowthrough material. Check pH and composition of sample and binding buffer.
The membrane protein is collected but is not pure (multiple bands on SDS polyacrylamide gel)	**Partial degradation of histidine tagged membrane protein by proteases:** Add protease inhibitors (use EDTA with caution- risk of removal of Ni-ions). **Contaminants have high affinity for nickel ions:** Wash before elution with binding buffer containing as high concentration of imidazole as possible, without causing elution of the tagged membrane protein.
No differences in yield between the screened detergents	The maximum binding capacity of His Mag Sepharose Ni could have been reached. Large amount of target protein is found in the flow-through material. Decrease the sample volume.
Precipitation, aggregation and loss of activity	Aggregation is a frequent problem with membrane proteins. Try to work in cold room and/or dilute buffers to decrease the concentration of NaCl.

7 Scaling up

Data obtained from detergent screening gives useful guidance for scale-up applications. For scale-up of purification, HisTrap™ columns 1 ml and 5 ml and Lab pack with Ni Sepharose are available.

8 Storage

Store the Membrane Protein Purification Kit at +4°C to +8°C.

9 Further information

For further information, visit http://www.gelifesciences.com/protein-purification, or www.gelifesciences.com, or contact your local GE Healthcare representative.

10 Ordering Information

Product	No. Supplied	Code No.
Membrane Protein Purification Kit	1	28-9805-82
MagRack 6	1	28-9489-64

Related products	No. Supplied	Code No.
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 × 1 ml	17-5247-01
HisTrap HP 5 ml	1 × 5 ml	17-5248-01
HisTrap HP 5 ml	5 × 5 ml	17-5248-02
Ni Sepharose High Performance	25 ml	17-5268-01
Ni Sepharose High Performance	100 ml	17-5268-02

Related products	No. Supplied	Code No.
His GraviTrap™	10 × 1 ml	11-0033-99
His SpinTrap™	50	28-4013-53
LMW Marker Kit	10 vials	17-0446-01
Full-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		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	Code No.
Purifying Challenging Proteins, Principles and Methods	28-9095-31
Recombinant Protein Purification Handbook	18-1142-75

Appendix

Binding Buffer composition with different imidazole concentrations

To obtain binding buffers of 20 mM phosphate, 500 mM NaCl, x mM imidazole, pH 7.4 containing the respective detergents, prepare buffers according to the table below. Add distilled water to final volume 4 ml.

Detergent	Phosphate buffer (stock)	2M imidazole 20 mM	2M imidazole 40 mM	2M imidazole 60 mM	2M imidazole 80 mM	Detergent stock
DDM 0.1%	500 µl	40 µl	80 µl	120 µl	160 µl	40 µl
DM 0.2%	500 µl	40 µl	80 µl	120 µl	160 µl	80 µl
LDAO 0.2%	500 µl	40 µl	80 µl	120 µl	160 µl	80 µl
FOS12 0.1%	500 µl	40 µl	80 µl	120 µl	160 µl	40 µl
$C_{12}E_8$ 0.1%	500 µl	40 µl	80 µl	120 µl	160 µl	40 µl
Cymal-5 0.2%	500 µl	40 µl	80 µl	120 µl	160 µl	80 µl
OG 1%	500 µl	40 µl	80 µl	120 µl	160 µl	200 µl

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Short Manual for the Membrane Protein Purification Kit

Binding buffers: 20 mM phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4, containing the respective detergent. Final volume will be 4.0 ml.

Binding buffer	Phosphate buffer (stock)	2M imidazole	Distilled water	Detergent stock
0.1% DDM	500 µl	40 µl	3.42 ml	40 µl
0.2% DM	500 µl	40 µl	3.38 ml	80 µl
0.2% LDAO	500 µl	40 µl	3.38 ml	80 µl
0.1% FOS12	500 µl	40 µl	3.42 ml	40 µl
0.1% $C_{12}E_8$	500 µl	40 µl	3.42 ml	40 µl
0.2% Cymal 5	500 µl	40 µl	3.38 ml	80 µl
1% OG	500 µl	40 µl	3.26 ml	200 µl

Elution buffers: 20 mM phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4, containing the respective detergent. Final volume will be 1.0 ml.

Elution buffer	Phosphate buffer (stock)	2M imidazole	Distilled water	Detergent stock
0.1% DDM	125 µl	250 µl	615 µl	10 µl
0.2% DM	125 µl	250 µl	605 µl	20 µl
0.2% LDAO	125 µl	250 µl	605 µl	20 µl
0.1% FOS12	125 µl	250 µl	615 µl	10 µl
0.1% $C_{12}E_8$	125 µl	250 µl	615 µl	10 µl
0.2% Cymal-5	125 µl	250 µl	605 µl	20 µl
1% OG	125 µl	250 µl	575 µl	50 µl

Suspension buffer (without detergent): 20 mM phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4: 1.25 ml Phosphate buffer (stock), 100 µl of 2 M imidazole. Add distilled water to final volume 10 ml.

Solubilization

1. Prepare seven tubes (1.5 ml), each with 900 µl of membrane, suspension and add 100 µl of the respective 10% (or 20%) detergent stock.
2. Mix by “end-over-end” mixing for 30 min at room temperature.
3. Clarify by centrifugation 50 000 × g for approximately 30 min. Collect the supernatants.

Purification

Magnetic bead preparation

1 Mix the medium slurry thoroughly by vortexing.
Dispense 200 µl of suspended magnetic beads into each of the seven tubes (1.5 ml).

Note: *Mix the medium slurry between each dispensation.*

2 Place the microcentrifuge tubes in MagRack 6.

3 Remove the storage solution.

Equilibration

4 Add 500 µl binding buffer (containing the respective detergent) into each of the seven tubes.

5 Resuspend the beads.

6 Remove the liquid.

Sample application

7 Add 1000 µl of sample. If the sample volume is less than 1000 µl, dilute to 1000 µl with the respective binding buffer.

8 Resuspend the beads and incubate for 30 minutes with slow "end-over-end" mixing.

9 Remove the liquid.

Washing

10 Add 500 µl binding buffer (containing the respective detergent). Resuspend the beads.

11 Remove the liquid.

12 Perform steps 10-11 three times.

Elution

13 Add 100 µl of elution buffer (containing the respective detergent). Resuspend the beads.

14 Remove and collect the eluted material. The eluate should contain most of the purified protein.

15 If desired, repeat the elution.