

P07601 - P07620 DUALrefold membrane protein refolding columns

Product Contents	DUALrefold membrane protein refolding columns 4 refolding columns Solution A (P07601 - P07610) - or - Solution B (P07611 - P07620) Solution C Solution S
Storage	Store columns at 4°C, do not freeze. Store Solutions A, B, C and S at -20°C for long-term storage or at 4°C for short-term storage. Avoid multiple freeze-thaw cycles.

Background The DUALrefold membrane protein refolding columns are designed for folding preparative amounts of membrane proteins from urea-solubilized inclusion bodies or other protein aggregates after optimal refolding conditions have been established using the DUALrefold membrane protein refolding kit (P07501).

Instructions This DUALrefold membrane protein refolding column set contains 4 large DUALrefold columns with a defined buffer/resin combination for preparative refolding of up to 5 mg of your membrane protein of interest.

In order to determine which DUALrefold column set provides the optimal conditions for your protein, you should first test all conditions using the DUALrefold membrane protein refolding kit (P07501).

For example, if you have determined that column #5 of the DUALrefold kit (P07501) is optimal for refolding your protein of interest, use the DUALrefold membrane protein refolding column set P07305 to refold preparative amounts of your membrane protein.

Optimal column determined using the DUALrefold membrane protein refolding kit	Corresponding DUALrefold large column
Column #1	P07601 Column (large) #1
Column #2	P07602 Column (large) #2
Column #3	P07603 Column (large) #3
Column #4	P07604 Column (large) #4
Column #5	P07605 Column (large) #5
Column #6	P07606 Column (large) #6
Column #7	P07607 Column (large) #7
Column #8	P07608 Column (large) #8
Column #9	P07609 Column (large) #9
Column #10	P076010 Column (large) #10
Column #11	P076010 Column (large) #11
Column #12	P076010 Column (large) #12
Column #13	P076010 Column (large) #13
Column #14	P076010 Column (large) #14
Column #15	P076010 Column (large) #15
Column #16	P076010 Column (large) #16
Column #17	P076010 Column (large) #17
Column #18	P076010 Column (large) #18
Column #19	P076010 Column (large) #19
Column #20	P076010 Column (large) #20

Protocol

- Perform all experiments in a 4° C cold room and keep samples on ice at all times unless indicated otherwise.
- Prepare a solution of solubilized inclusion bodies at a total protein concentration of 2 mg/ml.

Note

We recommend solubilizing the inclusion bodies by stirring in a buffer composed of 20 mM Tris-HCl, pH 7.0, 7 M GdnHCl (or 8 M urea), 10 mM DTT, 2 mM EDTA at room temperature for 4 hours. The solubilized material is then centrifuged at 125,000x g for 30 min to remove any insoluble material.

- To prepare the loading sample, mix 5 ml of the solubilized inclusion bodies with 5 ml of Solution A (supplied with P07601 - P07610) or with 5 ml of Solution B (supplied with P07611 - P07620).
- Incubate the loading sample at room temperature for 2 hr.
- Cut off the bottom tip of each column and let the storage buffer run through the column.
- Load 2.5 ml of the loading sample onto each column and wait until the sample has completely entered the column. If the flow stops, provide a little pressure by placing the cap onto the column. Discard the flow through.
- Elute the protein with 3.5 ml of Solution S.
- Collect the eluent, discard the columns.

- Incubate the eluent at 4°C for 2 hr.
- Mix 2 ml of Reagent C with each eluent and incubate the solution at 4°C for 2 hours to overnight.
- Remove any precipitate by centrifugation at 15,000 rpm for 5 min and collect the supernatant for analysis and purification of the refolded protein.

Protein purification after refolding

- The refolded protein can be purified by affinity, ion-exchange or gel filtration column chromatography.

Note

Some proteins are sensitive to low salt conditions; we recommend to dialyse the protein against a buffer containing 125 mM NaCl. Concentration by ultracentrifugation may also cause concentration of the detergent, leading to unwanted denaturation of the refolded membrane protein.

- Below, a protocol for purification using a Q-Sepharose column is given:
 - Dialyze the refolded protein against 40 volumes of 20 mM Tris-HCl, pH 8.5, 125 mM NaCl, 1 mM dodecyl maltoside, 2 mM DTT, 2mM EDTA at 4°C for 1-2 hours.
 - Remove any precipitates by centrifugation.
 - Equilibrate a Q-Sepharose column with the dialysis buffer.
 - Load the dialyzed protein solution onto the column.
 - Wash the column with 10 volumes of dialysis buffer.
 - Elute the protein with a salt gradient from 125 mM to 1 M in dialysis buffer.
 - Proceed with further purification / characterization as appropriate for your protein.

Support Please see www.dualsystems.com for support and protocols. Please direct support inquiries to support@dualsystems.com or call +41 44 738 50 00.

Research use This product is intended for research use only, not for diagnostic or therapeutic uses.

MSDS Please see the accompanying MSDS for safety and handling instructions. Observe good laboratory practice guidelines and wear gloves, laboratory coat and glasses when handling the product.

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