

P07501 DUALrefold membrane protein refolding kit

Product	DUALrefold membrane protein refolding kit
Contents	20 refolding spin columns (#1-20) Solution A, 200 µl (for use with columns #1-10) Solution B, 200 µl (for use with columns #11-20) Solution C, 2x 600 µl
Storage	Store at 4°C, do not freeze.

Background The DUALrefold membrane protein refolding kit is designed for folding membrane proteins from urea-solubilized inclusion bodies or other protein aggregates.

Instructions The kit is composed of 20 protein refolding spin columns and Solutions A, B and C. The 20 spin columns represent 20 different conditions with various detergents and lipids that form micelles and bicelles. The micellar and bicellar environments facilitate folding receptors, ion channels and other membrane proteins.

Once the optimal column for refolding your active protein is identified, preparative refolding columns with the specific condition (order numbers P07601 - P07620) are available for large-scale preparations of the folded membrane proteins.

For example, if you have determined that column #5 of the DUALrefold membrane protein refolding kit is optimal for refolding your membrane protein of interest, use P07605 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 2-3 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.

- Prepare Loading Samples A and B. To prepare Loading Sample A, mix 130 µl of the solubilized inclusion bodies with 130 µl of Solution A. To make Loading Sample B, mix 130 µl of the solubilized inclusion bodies with 130 µl of Solution B.
- Incubate Loading Sample A and Loading Sample B at room temperature for 2 hours.
- Pre-spin the columns at 3200 rpm for 30 sec using a standard bench-top microcentrifuge.
- Remove the bottom tips and caps of the columns.
- Place the columns into 1.5 ml-microcentrifuge tubes.
- Spin the columns at 1400 rpm for 2 min.
- Transfer each column into a fresh labeled 1.5-ml microcentrifuge tube.
- Load 25 µl of Loading Sample A per column onto columns #1 to 10.
- Load 25 µl of Loading Sample B per column onto columns #11 to 20.
- Spin the columns at 3200 rpm for 4 min.
- Discard the columns and incubate the eluent at 4°C for 2 hours.
- Add 50 µl of Reagent C to each eluent and incubate the solutions at 4°C overnight.
- Spin the solutions at 14,000 rpm for 5 min and collect the supernatant for activity measurement.

Analysis of the folded product

SDS-PAGE

- Use SDS-PAGE to check protein solubility for each of the samples obtained in the refolding process.
- Mix 10 µl of the supernatant containing the refolded protein with 10 µl water and 7 µl 4x SDS-PAGE loading buffer.

- Run a SDS-PAGE and check for the presence of your protein by Coomassie staining or Western Blotting using an antibody directed against your protein.

Activity assay

- Use any appropriate activity assay (e.g. a catalytic assay or a binding activity assay) to check your protein for activity.
- Dilute your protein 10 to 20 fold into the appropriate assay buffer (for a 100 µl assay volume, add 5 µl to 10 µl of your refolded protein solution).
- Run the assay.

No appropriate activity assay is available

- If no appropriate assay for protein activity is available, the chromatographic behavior of the protein can be used as an indicator for successful refolding.
- Use CD or SEC-MALS measurements to determine the folding state of your protein. Please note that CD or SEC-MALS measurements require purified protein in mg amounts.

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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