

## P07401 – P07410 DUALrefold soluble protein refolding columns (large)

<b>Product Contents</b>	DUALrefold soluble protein refolding columns (large) 4 large columns Solution A (5 ml) Solution B (8 ml) Solution C (15 ml)
<b>Storage</b>	Store at 4°C, do not freeze

**Background** DUALrefold refolding columns are effective and easy-to-use tools for protein refolding screens and preparative protein refolding. The columns are designed to produce active proteins from urea- or guanidine hydrochloride (GdnHCl)-solubilized inclusion bodies or other protein aggregates.

**Instructions** This DUALrefold soluble protein refolding column set contains 4 large DUALrefold columns with a defined buffer/resin combination for preparative refolding of up to 10 mg of your 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 soluble protein refolding kit (P07201).

For example, if you have determined that column #5 of the DUALrefold soluble protein refolding kit (P07201) is optimal for refolding your protein of interest, use the DUALrefold soluble protein refolding column set P07305 to refold preparative amounts of your protein.

Optimal column determined using the DUALrefold kit	Corresponding DUALrefold large column
Column #1	P07401 Column (large) #1
Column #2	P07402 Column (large) #2
Column #3	P07403 Column (large) #3
Column #4	P07404 Column (large) #4
Column #5	P07405 Column (large) #5
Column #6	P07406 Column (large) #6
Column #7	P07407 Column (large) #7
Column #8	P07408 Column (large) #8
Column #9	P07409 Column (large) #9
Column #10	P074010 Column (large) #10

## 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 for 4 columns, mix 5 ml of the solubilized inclusion bodies with 5 ml of Solution A.
- Incubate the sample for 5 minutes.
- Remove the bottom tip and cap of the column.
- Let the storage buffer run out of the column.
- Load 2.5 ml of the sample per 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 C.
- Collect the eluent and incubate at 4° C for 2-4 hours.
- Mix 2 ml of Solution B with the eluent and incubate at 4°C for 2 hours to overnight.

### Note

If solution B forms a precipitate during storage, warm it to room temperature to solubilize the precipitate, then cool it back to 4°C before use.

- Centrifuge the mixture at 14,000x g for 5 minutes.
- 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. 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, 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.

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**Support** Please see [www.dualsystems.com](http://www.dualsystems.com) for support and protocols. Please direct support inquiries to [support@dualsystems.com](mailto: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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