

## P07301 – P07310 DUALrefold soluble protein refolding spin columns

<b>Product Contents</b>	DUALrefold soluble protein refolding spin columns 10 spin columns Solution A Solution B
<b>Storage</b>	Store at 4°C, do not freeze

**Background** DUALrefold refolding spin 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 10 DUALrefold spin columns with a defined buffer/resin combination for preparative refolding 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 kit (P07201) is optimal for refolding your protein of interest, use P07305 to refold your protein.

Optimal column determined using the DUALrefold kit	Corresponding DUALrefold spin column
Column #1	P07301 Spin column (small) #1
Column #2	P07302 Spin column (small) #2
Column #3	P07303 Spin column (small) #3
Column #4	P07304 Spin column (small) #4
Column #5	P07305 Spin column (small) #5
Column #6	P07306 Spin column (small) #6
Column #7	P07307 Spin column (small) #7
Column #8	P07308 Spin column (small) #8
Column #9	P07309 Spin column (small) #9
Column #10	P073010 Spin column (small) #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 5 - 10 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.

- Pre-spin the column at 2100x g for 1 minute using a standard bench-top microcentrifuge.
- Remove the bottom tip and cap of the column.
- Place the column into 1.5 ml microcentrifuge tubes.
- Centrifuge the column at 1000x g for 2 minutes.
- Transfer the column into a clean, labeled 1.5 ml microcentrifuge tube.
- Mix 13 µl of the solubilized inclusion bodies with 13 µl of Solution A.
- Incubate the mixture for 5 minutes.
- Load 25 µl of the mixture onto the column.
- Centrifuge the column at 2100x g for 4 minutes.
- Discard the column and incubate the eluent at 4° C for 4 hours.
- Mix 50 µl of Solution B with the eluent and incubate at 4°C 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.

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<b>Support</b>	Please see <a href="http://www.dualsystems.com">www.dualsystems.com</a> for support and protocols. Please direct support inquiries to <a href="mailto:support@dualsystems.com">support@dualsystems.com</a> or call +41 44 738 50 00.
<b>Research use</b>	This product is intended for research use only, not for diagnostic or therapeutic uses.
<b>MSDS</b>	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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