

P07201 DUALrefold soluble protein refolding kit

Product Contents	DUALrefold soluble protein refolding kit 10 spin columns (numbered #1 - #10) Solution A, 200 µl Solution B, 600 µl
Storage	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. The DUALrefold kit contains 10 spin columns, each with a different resin/buffer composition. Each spin column provides a unique milieu for protein refolding and allows you to determine the optimal refolding conditions for your protein of interest.

Instructions Use the DUALrefold soluble protein refolding kit to determine which of the 10 spin columns is most effective at refolding your protein of interest to an active state. Once you have determined the optimal refolding conditions, use either DUALrefold spin columns (Product numbers P07301-P07310) or DUALrefold large columns (Product numbers P07401-P07410) to preparatively refold your protein of interest.

For example, if you have determined that column #5 of the DUALrefold kit is optimal for refolding your protein of interest, use P07305 to refold small amounts or P07405 to refold large amounts of your protein.

Optimal column determined using the DUALrefold kit	Corresponding DUALrefold spin column	Corresponding DUALrefold large column
Column #1	P07301 Spin column (small) #1	P07401 Column (large) #1
Column #2	P07302 Spin column (small) #2	P07402 Column (large) #2
Column #3	P07303 Spin column (small) #3	P07403 Column (large) #3
Column #4	P07304 Spin column (small) #4	P07404 Column (large) #4
Column #5	P07305 Spin column (small) #5	P07405 Column (large) #5
Column #6	P07306 Spin column (small) #6	P07406 Column (large) #6
Column #7	P07307 Spin column (small) #7	P07407 Column (large) #7
Column #8	P07308 Spin column (small) #8	P07408 Column (large) #8
Column #9	P07309 Spin column (small) #9	P07409 Column (large) #9
Column #10	P073010 Spin column (small) #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 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 columns at 2100x g for 1 minute using a standard bench-top microcentrifuge.
- Remove the bottom tips and caps of the columns.
- Place the columns into 1.5 ml microcentrifuge tubes.
- Centrifuge the columns at 1000x g for 2 minutes.
- Transfer each column into a clean, labeled 1.5 ml microcentrifuge tube.
- Mix 130 µl of the solubilized inclusion bodies with 130 µl of Solution A.
- Incubate the mixture for 5 minutes.
- Load 25 µl of the mixture onto each column
- Centrifuge the columns at 2100x g for 4 minutes.
- Discard the columns and incubate the eluent at 4° C for 2-4 hours.
- Mix 50 µl of Solution B with each 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 mixtures at 14,000x g for 5 minutes
- Collect the supernatant for analysis or purification of the folded protein.

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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