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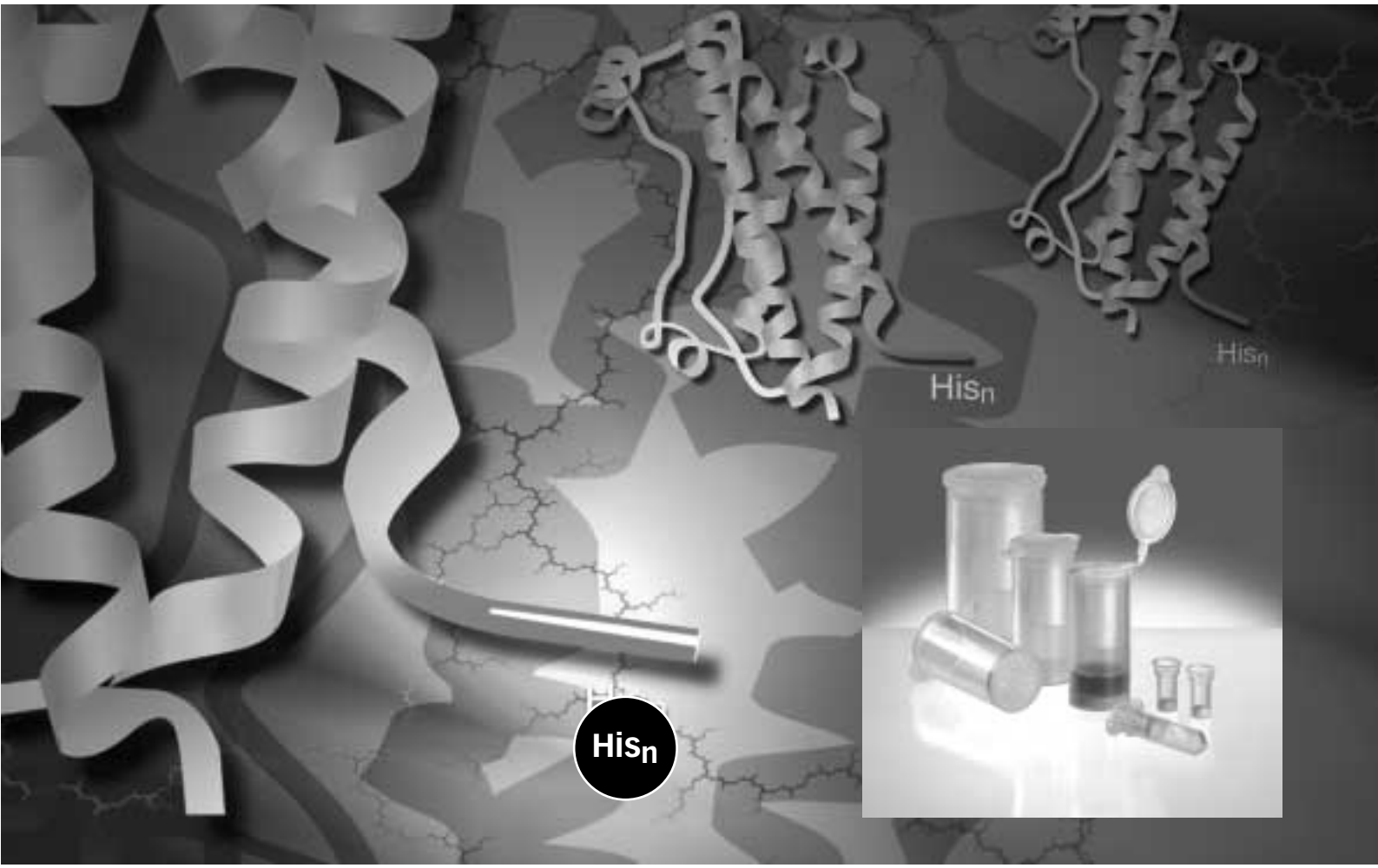

Vivapure Metal Chelate Mega spin columns

Technical data and operating instructions.
For *in vitro* use only.

Vivapure Metal Chelate Mega spin columns - for the purification of proteins with poly-histidine tags

Storage conditions

Vivapure Metal Chelate Mega spin columns can be stored at room temperature. They have a guaranteed shelf life of 12 months from the date of purchase.

Introduction

Vivapure Metal Chelate Mega spin columns represent a new generation of Immobilized Metal Affinity Chromatography (IMAC) purification devices, which simply can be used in a centrifuge. IMAC is a common and effective tool for the purification of poly-histidine tagged proteins. The method is based on the ability of some proteins to bind to immobilized metal ions. Especially strong interactions take place with the commonly used poly-histidine $(His)_6$ tag with six consecutive histidine residues. Using the IMAC principle, poly-histidine tagged proteins can be concentrated to a high degree of purity even from cell lysates or culture supernatants.

The Vivapure Metal Chelate Mega spin columns have covalently bound IDA (iminodiacetic acid) groups on the membrane. The IDA groups can be loaded with different metal ions depending on the particular application. We suggest using nickel (Ni^{++}), cobalt (Co^{++}), copper (Cu^{++}) or zinc (Zn^{++}) ions, but also different metal ions can easily be immobilized on the membrane. Proteins engineered with poly-histidine tags passing through the prepared membrane are preferentially bound. These bound proteins can be easily eluted from the membrane using buffers with varying concentrations of imidazole.

Vivapure Metal Chelate Mega spin columns are designed to simplify the chromatographic steps normally associated with IMAC. They are ideally suited for the quick and convenient purification of poly-histidine tagged proteins (approx. 18-20 mg).

Technical assistance

For more information, please contact the Vivascience Support Center.

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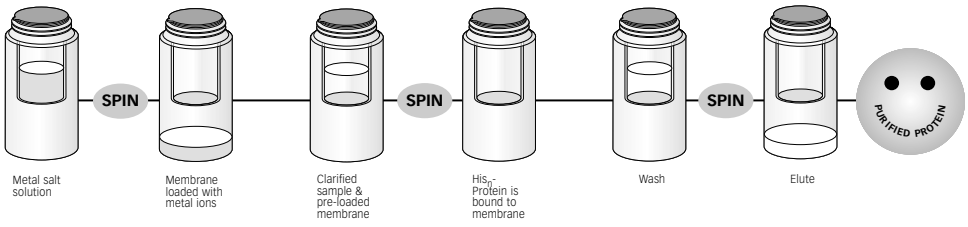
Vivapure Metal Chelate Mega spin columns	
Cat. No.	VS-MC75MH02
Vivapure Metal Chelate Mega spin columns	2
250 ml centrifuge bottles - standard caps	2
Instruction Manual	1

Specifications	
Max. volume per centrifuge run	75 ml
Recommended centrifugation speed	500 x g - 1000 x g (please refer to protocol)
Binding capacity	18-20 mg
Materials of construction	
Vivapure Metal Chelate Mega spin columns	Polypropylene
Clarification Mega spin columns	Polypropylene
Collection tubes	Polycarbonate

Handling overview

There are a number of various expression systems for poly-histidine tagged proteins available.

This protocol addresses protein purification from bacterial expression systems. The procedure may be adapted to other related expression systems, e.g. yeast or eukaryotic cells.



Protocol for Vivapure Metal Chelate Mega spin columns

Additional equipment required:

Hardware: Any centrifuge with swing-out rotor that can accommodate 250 ml centrifuge BOTTLES and can spin samples at speeds up to 2000 x g.

Buffers and aqueous metal salt solutions: Please refer to the section "Recommended buffers" and "Recommended aqueous metal salt solutions".

Optional devices for sample concentration after elution: Vivaspin 20 (e.g. 10 kDa MWCO, PES membrane, Vivascience Catalog No. V2001) or Vivacell 70 concentrators fitting in the Vivapure Mega bottle (e.g. 10 kDa MWCO, PES membrane, Vivascience Catalog No. VS6002). For further information, see Vivascience Ultrafiltration Catalog or www.vivascience.com.

Sample preparation

Prepare the cell lysates according to your standard protocol (sodium phosphate is recommended as buffer system for cell lysis) and subsequently continue with the step "Sample clarification" described below.

For more detailed information on the preparation of cell lysates, please refer to general information sources like "Current Protocols in Molecular Biology" by Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J. G. Seidman, Kevin Struhl.

Sample clarification

To avoid clogging of the Vivapure Metal Chelate Mega spin column, the original sample should be centrifuged at high g-force and pre-filtered before it is loaded onto the Vivapure Mega spin column.

1. Spin sample for 10-15 min at high g-force to pellet insoluble particles.

2. If the sample is still cloudy, we recommend to filter the sample with a Minisart syringe filer (1.2 µm, order No. 17593k).

Store the clarified sample on ice or under appropriate conditions and pre-load the membrane of the Vivapure Metal Chelate Mega spin column with metal ions (next step).

Membrane pre-loading with metal ions:

As described above, various metal ions can be bound to the chelate membrane. We recommend using nickel (Ni^{++}), cobalt (Co^{++}), copper (Cu^{++}) or zinc (Zn^{++}) ions for purification. Nevertheless, depending on the particular application, even better results may be achieved with other metal ions. See also section "Recommended aqueous metal salt solutions".

1. Prepare the 0.5 M salt solution(s) of your choice as described above.
2. Pre-wet the Vivapure Metal Chelate Mega spin column with 20 ml distilled water. Spin for 3 min at 500 x g and discard the flow-through.
3. Load 10 ml of a 0.5 M aqueous metal salt solution. Spin for 3 min at 500 x g and discard the flow-through. Repeat this step.
4. Fill in 20 ml distilled water and spin for 3 min at 500 x g to remove unbound metal ions. Repeat this step.

Now the Vivapure Metal Chelate Mega spin column is ready for sample loading.

Sample loading and purification

1. Equilibrate the membrane with 20 ml equilibration buffer. Spin for 5 min at 500 x g and discard the flow-through. Repeat this step.
2. Load up to 75 ml sample solution onto the membrane. Centrifuge for 5 min at 1000 x g. For sample volumes higher than 75 ml, the Vivapure Metal Chelate Mega spin column can be re-loaded to bind your complete sample as long as the membrane capacity is considered.
3. Wash the membrane with 20 ml washing buffer. Spin for 5 min at 1000 x g and discard the flow-through. Repeat this step.
   Note: An optional third washing step might be included to increase purity.
4. Pipette 10 ml elution buffer onto the membrane to elute the protein. Spin for 3 min at 1000 x g. The flow-through contains the purified protein.
5. A second and third elution step with 10 ml elution buffer may be necessary to elute all of the desired protein.

Optional concentration or buffer exchange after elution:

For subsequent applications, is often useful to further concentrate the eluate or to exchange the buffer in the eluate. Both applications can be easily and conveniently performed using Vivaspin 20 or Vivacell 70 (see above).

Recommended aqueous metal salt solutions:

Pre-loading with metal ions, choice of optimal metal ion for your purification.

→ See Table 1 below

Vivapure Metal Chelate Mega spin columns were specifically designed to allow you to choose the metal ions to be immobilized on the membrane. If there is little prior knowledge about the purification of your poly-histidine tagged target protein, we recommend to start with nickel (Ni^{++}), cobalt (Co^{++}), copper (Cu^{++}) or zinc (Zn^{++}) ions.

Alternatively, you can also use Vivapure Metal Chelate Mini spin columns (Order No. VS-MC01MC12) to screen for the optimal metal ion. The Mini columns allow you to test the parallel purification of small amounts of protein.

Table 1: Recommended aqueous solutions of Ni^{++}, Co^{++}, Cu^{++} or Zn^{++} ions for the step "Membrane pre-loading with metal ions"

Ni^{++}	0.5 M Nickel sulphate (e.g. $NiSO_4 \cdot 6H_2O$) in deionized water
Co^{++}	0.5 M Cobalt chloride (e.g. $CoCl_2 \cdot 6H_2O$) in deionized water
Cu^{++}	0.5 M Copper sulphate (e.g. $CuSO_4$ or $CuSO_4 \cdot 5H_2O$) in deionized water
Zn^{++}	0.5 M Zinc chloride (e.g. $ZnCl_2$) in deionized water

Recommended buffers for equilibration, washing and elution:

→ See Table 2 below

As mentioned above, expression systems for proteins with poly-histidine tags are highly diverse. Therefore, the described purification buffers should be considered only as guidelines. For the best performance and recovery, we recommend optimization of conditions for the individual target protein.

Contaminating proteins in the eluate can be reduced by varying the imidazole concentration in the washing buffer. If the poly-histidine tagged protein cannot be detected after elution with 250 mM imidazole, the imidazole concentration of the elution buffer should be increased. Depending on the elution conditions, leaching of the metal ions from the membrane may occur.

We recommend the following buffers for purifying the protein of interest under native conditions - see Table 2.

Table 2: Buffer recommendations for purification under native conditions*

Equilibration buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0*
Washing buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0*
Elution buffer: 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0*

* Under denaturing conditions 8 M urea may be added.

Ordering Information

Cat Number	Vivapure Spin Columns	Spin Columns
VS-PA01PA24	Protein A Mini	24
VS-MC01MC12	Metal Chelate Mini	12
VS-MC20MH04	Metal Chelate Maxi	4
VS-MC75MH02	Metal Chelate Mega	2

Cat Number	Vivapure Kits	Spin Columns
VS-MC01MCOP	Metal Chelate Optimization Kit	12
VS-PC01EPPC	Epoxy Protein Coupling Kit	12
VS-IX01QHGP	Acidic Protein Purification Kit Q Mini H	8
VS-IX20QHGP	Acidic Protein Purification Kit Q Maxi H	4
VS-IX01SHGP	Basic Protein Purification Kit S Mini H	8
VS-IX20SHGP	Basic Protein Purification Kit S Maxi H	4
VS-IX01DMDR	DNA Removal Kit D Mini M	12
VS-IX20DMDR	DNA Removal Kit D Maxi M	6

Cat Number	Vivapure Ion Exchange Mini Spin Columns	Spin Columns	Centrifuge Tubes
VS-IX01ST16	Vivapure Mini H Starter Kit (4 of each ion exchange class)	16	32
VS-IX01CL24	Vivapure C Mini L	24	48
VS-IX01CM24	Vivapure C Mini M	24	48
VS-IX01CH24	Vivapure C Mini H	24	48
VS-IX01DL24	Vivapure D Mini L	24	48
VS-IX01DM24	Vivapure D Mini M	24	48
VS-IX01DH24	Vivapure D Mini H	24	48
VS-IX01QL24	Vivapure Q Mini L	24	48
VS-IX01QM24	Vivapure Q Mini M	24	48
VS-IX01QH24	Vivapure Q Mini H	24	48
VS-IX01SL24	Vivapure S Mini L	24	48
VS-IX01SM24	Vivapure S Mini M	24	48
VS-IX01SH24	Vivapure S Mini H	24	48

Cat Number	Vivapure Ion Exchange Maxi Spin Columns	Spin Columns	Centrifuge Tubes
VS-IX20CM08	Vivapure C Maxi M	8	16
VS-IX20CH08	Vivapure C Maxi H	8	16
VS-IX20DM08	Vivapure D Maxi M	8	16
VS-IX20DH08	Vivapure D Maxi H	8	16
VS-IX20QM08	Vivapure Q Maxi M	8	16
VS-IX20QH08	Vivapure Q Maxi H	8	16
VS-IX20SM08	Vivapure S Maxi M	8	16
VS-IX20SH08	Vivapure S Maxi H	8	16

Cat Number	Vivapure Ion Exchange Mega Spin Columns	Spin Columns	Centrifuge Tubes
VS-IX75QH02	Vivapure Q Mega H	2	2
VS-IX75DH02	Vivapure D Mega H	2	2
VS-IX75SH02	Vivapure S Mega H	2	2
VS-IX75CH02	Vivapure C Mega H	2	2

For more information on related products, please refer to the:

- Vivascience Ultrafiltration Catalog - Vivaspin
- Vivapure® Protein A Mini spin column brochure
- Vivascience Cell Culture Catalog
- Vivapure® Catalog for kits and devices for protein purification
- Vivapure® Epoxy Protein Coupling kit brochure

For current information and application notes, please visit us at www.vivascience.com

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