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invitrogen bead separations

Cat. no. 105.13D
105.14D

Rev. no. 002

Dynabeads® SCX

For research use only.

INDEX

1. PRODUCT DESCRIPTION

1.1 Intended Use

Dynabeads SCX exploit differences in net charge to separate proteins and peptides; bead-bound charged groups reversibly adsorb oppositely charged molecules.

These strong cationic exchanger (SCX) beads are ideal for:

- capture and purification of proteins/peptides prior to mass spectrometry (MS) analysis.
- fractionation of complex protein/peptide mixtures (e.g. serum, plasma, CSF, urine, cell lysates) prior to MS analysis, 2D gel electrophoresis or HPLC analysis.
- peptide profiling and biomarker screening prior to MS analysis.
- removal of detergents

Capture and fractionation can also be automated as required.

1.2 Material Supplied

Product No.	Volume	Bead diameter	Supplied in	Concentration	Monodispersity
105.13D	2 ml	1.0 µm	0.1M HEPES pH 8.0, with 0.02% NaN_3 (preservative)	12.5 mg beads/ml solution	C.V < 5%
105.14D	20 ml				

Each mg of Dynabeads SCX can adsorb approx. 80 µg of Lysozyme (MW 14.4 kDa).

1.3 Additional Materials Required

- Magnet: see www.invitrogen.com/magnets-selection for magnet recommendations
- Buffers and solutions (see individual protocols)

Buffers and Solutions

The following buffers are examples for use with Dynabeads SCX but alternative adsorption and/or desorption solutions can be used. All reagents should be freshly prepared and of analytical grade.

The choice of buffers and solutions will depend upon the proteins and/or peptides to be isolated.

For efficient adsorption, the ionic strength of the adsorption buffer must be low and the pH at least one pH unit below the pI of the molecule to be bound (Fig. 1).

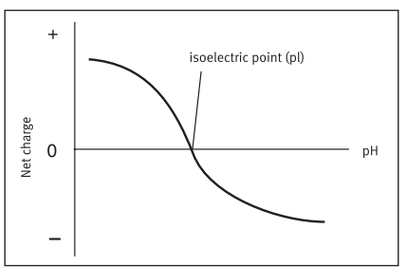

Fig. 1: Titration curve showing how the net charge of a protein or peptide varies with increasing pH.

Optional Use of Detergents

The use of low concentrations of non-ionic detergents (e.g 0.01 % Tween 20 or 8mM n-octylglucoside) can improve the handling of Dynabeads SCX. Do not introduce detergents that might interfere with downstream analyses (e.g. mass spectrometry or use of a liquid chromatography (LC) column).

- **Pre-Loading Solution (high ionic strength)**

  50 mM sodium phosphate pH 8.0, 1M NaCl or 50 mM HEPES pH 8.0, 1M NaCl

- **Adsorption Solution pH 8.0**

  50 mM sodium phosphate pH 8.0, 10 mM NaCl, or 50 mM HEPES pH 8.0, 10 mM NaCl

- **Adsorption Solution pH 3.0**

  20 mM citric acid, pH 3.0, 10 mM NaCl

- **Adsorption/Desorption Solutions**

  For desorption using increasing pH or for desorption/adsorption using multiple pH conditions the following solutions can be used: The optimal buffer or solution will depend upon the proteins and/or peptides to be isolated.

  50 mM lactic acid buffer pH 4.0

  50 mM acetic acid pH 5.0 – 5.5

  50 mM MES pH 6.0

  50 mM sodium phosphate buffer pH 7.0

  50 mM HEPES pH 8.0

  20 mM ammonium acetate pH 9.5

  50 mM ammonium acetate pH 9.5

  For adsorption the solutions can include low salt concentration, e.g. 10mM NaCl.

- **Desorption Solutions**

  For desorption using increasing salt concentrations (optimize salt concentrations for specific samples). pH 8.0 is one example.

  50 mM sodium phosphate pH 8.0, 50 mM NaCl

  50 mM sodium phosphate pH 8.0, 100 mM NaCl

  50 mM sodium phosphate pH 8.0, 150 mM NaCl

  50 mM sodium phosphate pH 8.0, 200 mM NaCl

  50 mM sodium phosphate pH 8.0, 250 mM NaCl

2. FRACTIONATION OF PROTEIN/PEPTIDE MIXTURES

Optimize the quantity of beads used for each individual application by titration. Scale elution volumes accordingly to avoid unnecessary sample dilution.

2.1 Pre-Loading of Dynabeads

Load Dynabeads with counter-ions and equilibrate in Adsorption Solution before use.

1. Resuspend Dynabeads thoroughly to obtain a homogeneous suspension.
2. Transfer 40 µl (0.5 mg) beads to a tube.
3. Place the tube on the magnet for 1-2 mins.
4. Discard the supernatant by aspiration with a pipette while the tube remains on the magnet.
5. Remove the tube from the magnet.
6. Add 100 µl high ionic strength Pre-Loading Solution (see section 2.2) along the inside of the tube where the beads are collected and resuspend.
7. Place the tube on the magnet for 1-2 mins and discard the supernatant.
8. Remove the tube from the magnet. Add 100 µl Pre-Loading Solution and resuspend.
9. Repeat steps 7 and 8 for a total of two washes.

2.2 Equilibration

For fractionation using multiple pH adsorption reactions, see 2.2.1.

1. Place the tube on the magnet for 1-2 mins and remove the supernatant.
2. Remove the tube from the magnet.
3. Add 100 µl Adsorption Solution along the inside of the tube where the beads are collected and resuspend.
4. Place the tube on the magnet for 1-2 mins and discard the supernatant.
5. Remove the tube from the magnet. Add 100 µl Adsorption Solution and resuspend.
6. Place the tube on the magnet for 1-2 mins and discard the supernatant.
7. Remove the tube from the magnet, add 100 µl Adsorption Solution and resuspend.

The Dynabeads are now equilibrated and ready for use.

2.2.1 Equilibration for Fractionation using Multiple pH Adsorption Reactions

Pre-load enough Dynabeads for multiple adsorption reactions (see section 2.1).

1. Divide the pre-loaded Dynabeads into the same number of aliquots as there are adsorption reactions (0.5 mg beads per vial).
2. Place each tube containing pre-loaded beads on the magnet for 1-2 mins and discard the supernatants.
3. Remove the tubes from the magnet. Add 100 µl of the appropriate Adsorption Solution to each adsorption reaction (e.g. 20 mM citric acid pH 3, 10 mM NaCl; 50 mM acetic acid pH 5.0, 10 mM NaCl; 50 mM sodium phosphate buffer pH 7.0, 10 mM NaCl) along the inside of the tube where the beads are collected and resuspend.
4. Place the tubes on the magnet for 1-2 mins and discard the supernatants.
5. Remove the tubes from the magnet, add 100 µl of the appropriate Adsorption Solution (see step 3) to each tube and resuspend.
6. Place the tubes on the magnet for 1-2 mins and discard the supernatants.
7. Remove the tubes from the magnet, add 100 µl of the appropriate Adsorption Solution (see step 3) and resuspend.

The Dynabeads are now equilibrated and ready for use.

2.3 Sample Preparation

Prepare your starting sample (serum, plasma, CSF, cell lysate or other protein-containing material).

2.4 Fractionation by Step-wise Elution by Increasing pH

Protein/Peptide Adsorption

1. Add your sample containing an appropriate amount of protein/peptide (approx. 40 µg protein to 0.5 mg beads) to the vial containing the washed and equilibrated Dynabeads from 2.2. Adjust the total volume to 200 µl with Adsorption Solution (e.g. 20 mM citric acid pH 3.0, 10 mM NaCl). Mix using a pipette.
2. Leave at room temperature for 2 mins to allow proteins/peptides to adsorb to the beads.
3. Place the tube on the magnet. When the beads are at the tube wall and the liquid is clear, discard the supernatant.
4. Remove the tube from the magnet, add 200 µl Adsorption Solution and mix.
5. Separate the beads from the buffer using a magnet and discard the supernatant.
6. Repeat steps 4 and 5 twice for a total of three washes.

Protein/Peptide Desorption

Fractionate the protein/peptide mixture by eluting stepwise with solutions of increasing pH.

1. Resuspend the beads in 20 µl Desorption Solution (e.g 50 mM lactic acid buffer pH 4.0) and incubate for 2 mins at room temperature.
2. Place the tube on the magnet and remove the eluate.
3. Repeat steps 1 and 2 as many times as needed using different Desorption Solutions with increasing pH (see section 1.3).

Each eluate now contains a different fraction of proteins from the original sample.

2.5 Fractionation by Step-wise Elution by Increasing Salt Concentration

Protein/Peptide Adsorption

1. Add your sample containing an appropriate amount of protein/peptide (approx. 40 µg protein to 0.5 mg beads) to the vial containing the washed and equilibrated Dynabeads from 2.2. Adjust the total volume to 200 µl with Adsorption Solution (e.g. 50 mM sodium phosphate pH 8.0, 10 mM NaCl). Mix using a pipette.
2. Leave at room temperature for 2 mins to allow proteins/peptides to adsorb to the beads.
3. Place the tube on the magnet. When the beads are at the tube wall and the liquid is clear, discard the supernatant.
4. Remove the tube from the magnet, add 200 µl Adsorption Solution and mix.
5. Separate the beads from the buffer with a magnet and discard the supernatant.
6. Repeat steps 4 and 5 twice for a total of three washes.

Protein/Peptide Desorption

Fractionate the protein/peptide mixture by eluting stepwise with solutions of increasing salt concentration.

1. Resuspend the beads in 20 µl Desorption Solution (e.g. 50 mM sodium phosphate pH 8.0, 50 mM NaCl) and incubate for 2 mins at room temperature.
2. Place the tube on the magnet and remove the eluate.
3. Repeat steps 1 and 2 as many times as needed using different Desorption Solutions of increasing salt concentrations (see section 1.3).

Each eluate now contains a different fraction of proteins/peptides from the original sample.

Desalting Protocol

If necessary, desalt fractions containing salt using Dynabeads® RPC Protein or Dynabeads® RPC 18.

2.6 Fractionation using Multiple pH Adsorption Reactions

Pre-load enough Dynabeads SCX for multiple adsorption reactions, see 2.1.

Protein/Peptide Adsorption

1. Add your sample containing an appropriate amount of protein/peptide (approx. 40 µg protein/peptide per aliquot) to the tubes containing the washed and equilibrated Dynabeads from 2.2.1. Adjust the total volumes to 200 µl with the appropriate Adsorption Solutions. Mix using a pipette.
2. Leave at room temperature for 2 mins to allow proteins/peptides to adsorb to the beads.
3. Place the tubes on the magnet. When the beads are at the tube wall and the liquid is clear, discard the supernatants.
4. Remove the tubes from the magnet, add 200 µl of the appropriate Adsorption Solutions and mix.
5. Separate the beads from the buffer using a magnet and discard the supernatants.
6. Repeat steps 4 and 5 twice for a total of three washes.

Protein/Peptide Desorption

1. Resuspend the beads in 20 µl Desorption Solution (e. g. 50 mM ammonium acetate pH 9.5) and incubate for 2 mins at room temperature.
2. Place the tubes on the magnet and remove the eluates.

Each eluate contains a different fraction of proteins/peptides from the original sample.

3. SERUM PEPTIDE PROFILING

The following protocol has been optimized for peptide profiling using serum. Using serum diluted in the proportions described was optimal with regards to ion peak number and reproducibility. For reproducible serum profiling use enough buffer to obtain the desired (and identical) pH for all samples during adsorption.

Load Dynabeads with counter-ions and equilibrate in Adsorption/Washing Buffer before use.

3.1 Pre-Loading

1. Resuspend the Dynabeads thoroughly to obtain a homogeneous suspension.
2. Transfer 20 µl (0.25 mg) Dynabeads to a tube.
3. Place the tube on the magnet for 1-2 mins.
4. Remove the supernatant by aspiration with a pipette while the tube remains on the magnet.
5. Remove the tube from the magnet.
6. Add 50 µl high ionic strength Pre-Loading Buffer (50 mM HEPES pH 8, 1 M NaCl, 8 mM n-octylglucoside) along the inside of the tube where the beads are collected and resuspend.
7. Place the tube on the magnet for 1-2 mins and remove the supernatant.
8. Remove the tube from the magnet, again add 50 µl Pre-Loading Buffer and resuspend.
9. Repeat steps 7 and 8 for a total of two washes.

3.2 Equilibration

1. Place the tube on the magnet for 1-2 mins and remove the supernatant.
2. Remove the tube from the magnet.
3. Add 50 µl Adsorption/Washing Buffer (20 mM Citric acid pH 3, 10 mM NaCl, 8 mM n-octylglucoside) along the inside of the tube where the beads are collected and resuspend.
4. Place the tube on the magnet for 1-2 mins and remove the supernatant.
5. Remove the tube from the magnet, again add 50 µl Adsorption/Washing Buffer and resuspend.
6. Place the tube on the magnet for 1-2 mins and remove the supernatant.
7. Remove the tube from the magnet, add 30 µl Adsorption/Washing Buffer and resuspend.

The Dynabeads are now equilibrated and ready for use.

3.3 Peptide and Protein Adsorption

1. Add 10 µl serum to the vial containing the equilibrated Dynabeads from 3.2. Mix using a pipette.
2. Leave at room temperature for 2 mins to allow peptides to adsorb to the beads.
3. Place the tube on the magnet. When the beads are at the tube wall and the liquid is clear, discard the supernatant.
4. Remove the tube from the magnet, add 200 µl Adsorption/Washing Buffer and mix.
5. Again separate the beads from the buffer using the magnet and discard the supernatant.
6. Repeat steps 4 and 5 twice for a total of three washes.

3.4 Peptide and Protein Desorption

1. Resuspend the beads in 7.5 µl 50 mM ammonium acetate pH 9.5, 8 mM n-octylglucoside and incubate for 2 mins at room temperature.
2. Place the tube on the magnet and remove the eluate.

3.5 MALDI-TOF Mass Spectrometry Analysis

3.5.1 Detection of peptides < 4000 Da

To detect peptides < 4000 Da run the mass spectrometer in reflector positive mode and spot as follows (see 5.3.5):

1. Mix aliquots (1 µl) of eluates with 2 µl of the MALDI matrix µ-cyano-4-hydroxy-cinnamic acid (supplied by Agilent in 36 % methanol/56 % acetonitrile).
2. Deposit 0.5 µl of the eluate/matrix mixture on a stainless steel MALDI target plate.
3. Run MALDI-TOF mass spectrometry analysis,e.g. using a 4700 MALDI-TOF/TOF mass spectrometer (Applied Biosystems) data can be acquired automatically through the instrument software programmed to acquire 2000 shots per spectrum from a total of 40 different locations in the spot (i.e. 50 shots per location).

3.5.2 Detection of larger peptides and proteins

For the detection of larger peptides and proteins run the mass spectrometer in linear mode and spot an acidified matrix/eluate mixture (see 3.5.3):

1. Mix aliquots (1 µl) of eluates with 2 µl of the MALDI matrix α-cyano-4-hydroxy-cinnamic acid (dissolved in 36 % methanol, 56 % acetonitrile, 0.8 % TFA).
2. Deposit 0.5 µl of the eluate/matrix mixture on a stainless steel MALDI target plate.
3. Run MALDI-TOF mass spectrometry analysis,e.g. using a 4700 MALDI-TOF/TOF mass spectrometer (Applied Biosystems) data can be acquired automatically through the instrument software programmed to acquire 3000 shots per spectrum from a total of 60 different locations in the spot (i.e. 50 shots per location).

3.5.3 The pH of the spotting solution influences ion detection by mass spectrometry

The previous sections describe two alternative α-cyano-4-hydroxy-cinnamic acid matrix solutions. Adding 0.8 % TFA to the matrix solution will typically decrease the pH of the eluate/matrix spotting mixture from pH 4 – 4.5 to approximately pH 1.5. This acidification of the eluate/matrix mixture significantly alters mass spectra in favour of the detection of higher mass ions (Fig. 5)(8). Adding TFA is therefore recommended when analyzing using linear mode.

4. AUTOMATED PROTOCOLS

Dynabeads SCX are ideal for automated applications due to their small size, low sedimentation rate and high magnetic mobility. Magnetic separation and handling can be easily automated on a wide variety of platforms.

Please contact Invitrogen Dynal for further technical information (see contact details).

6. GENERAL INFORMATION

Invitrogen Dynal AS complies with the Quality System Standards ISO 9001:2000 and ISO 13485:2003.

6.1 Storage and Stability

When stored unopened at 2–8°C, this product is stable until the expiration date stated on the label.

Store opened vials at 2–8°C and avoid bacterial contamination. Do not freeze the product. Keep Dynabeads in liquid suspension during storage and all handling steps, as drying will result in reduced performance. Resuspend well before use. Store the beads at a greater pH than 6. Do not store in water.

6.2 References

1. Simpson RJ. (2003). Proteins and proteomics, a laboratory manual. Cold Spring Harbor Laboratory Press (ISBN 0-87969-553-6)

6.3 Related Dynabeads Products

A comprehensive range of Dynabeads for use in proteomic workflows are available.

Cat. no.	Product Description
105.11D/12D	Dynabeads® WCX Weak cation exchange 1 µm beads for fractionation of protein and peptide samples.
105.15D/16D	Dynabeads® SAX Strong anion exchange 1 µm beads for fractionation of protein and peptide samples.
102.11D/12D	Dynabeads® RPC 18 Hydrophobic 1 µm beads for fractionation of peptide samples.
102.16D/17D	Dynabeads® RPC Protein Hydrophobic 1 µm beads for fractionation of large peptide and protein samples.

6.4 Warnings and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use.

Follow appropriate laboratory guidelines.

This product contains 0.02% sodium azide (NaN_3) as a preservative. Sodium azide is toxic if ingested. Avoid pipetting by mouth. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build-up.

Material Safety Data Sheet (MSDS) is available at http://www.invitrogen.com.

Certificate of Analysis/Compliance is available upon request.

6.5 Trademarks and Patents

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6.8 Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the vial and outer labels for the duration of the stated shelf life. Invitrogen Dynal's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Invitrogen Dynal's expense, of any products which shall be defective in manufacture, and which shall be returned to Invitrogen Dynal, transportation prepaid, or at Invitrogen Dynal's option, refund of the purchase price.

Claims for merchandise damaged in transit must be submitted to the carrier.

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5. OPTIMIZATION PARAMETERS

Problem	Possible Cause	Suggested Solutions
Poor adsorption of proteins to beads	Ionic interaction is not strong enough	Decrease the ionic strength of the adsorption solution
	pH during adsorption is higher than the pI of the proteins of interest	Decrease the pH of the solution used during adsorption
Difficulty eluting proteins from beads	Ionic interaction is too strong	Increase the NaCl concentration used during elution
		Increase the pH used during elution
Poor separation of proteins into fractions	Carry-over between fractions	Include a wash step between each elution step
	Proteins of interest have similar net charges	Optimize pH steps or NaCl concentrations used during elution

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