DYNAL BIOTECH

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Dynabeads® TALON™

For research use only

Dynabeads® TALON™ are magnetizable beads developed for the isolation of recombinant histidine-tagged proteins. The product employs cobalt-based Immobilized Metal Affinity Chromatography (IMAC) using Dynabeads on which the BD TALON™ chemistry has been immobilized. The BD TALON technology is licenced from BD Biosciences Clontech, USA.

Dynabeads TALON bind histidine-tagged proteins with an enhanced selectivity. Bound proteins can be used directly in downstream applications or the recombinant protein can be be eluted off the beads. Elution conditions are less stringent than Ni-based IMAC, thus ensuring fully functional isolated proteins.

For more information about the BD TALON chemistry visit 'www.bdbiosciences.com'. For more Dynabeads product information visit 'www.dynalbiotech.com'.

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1. PRODUCT DESCRIPTION

Dynabeads® TALON™ are uniform, superparamagnetic polystyrene beads, 1 µm in diameter, coupled with highly specific BD TALON chemistry. The BD TALON technology is comprised of a tetradentate metal chelator in which four of cobalt's six coordination sites are occupied. The imidazole rings of histidine residues present in a poly histidine peptide chain are able to occupy the two remaining coordination sites, resulting in protein binding.

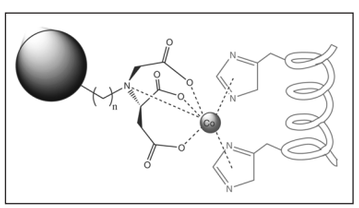

Fig. 1: Dynabeads TALON with a bound alfa-helix histidine-tag.

Dynabeads TALON is the ideal product for purifying recombinant histidine-tagged proteins often expressed in *E. coli*. Magnetic bead-based technology makes the purification quick and easy. Protocols for the purification of histidine-tagged proteins using other metal based IMAC technologies can easily be adapted for cobalt based IMAC. However, some optimisation may be required.

- Dynabeads TALON are supplied in 20% EtOH at a concentration of 40 mg beads per ml solution.
- By using 1 mg beads approximately 10 µg of a 30 kDa histidine-tagged protein is eluted after solid-phase isolation.
- Diameter: 1.1 µm (C.V. max 5%).

Additional material required

- Magnet particle concentrator for manual protocol (Dynal MPC®-S is recommended for 20 µl – 2 ml sample)
- Mixing device allowing rotation of tubes (e.g. a roller or Dynal® Sample Mixer)
- Buffers:
  The following buffers are recommended for use with Dynabeads TALON in the isolation protocol described in section 2.2 below. Alternative binding and/or washing buffers may also be used for isolation of your specific recombinant protein.

TALON™ Binding and Washing Buffer

50 mM NaP, pH 8,0
300 mM NaCl
0,01% Tween®-20

TALON™ Elution Buffer

150 mM Imidazole
50 mM NaP pH 8,0
300mM NaCl
0,01% Tween®-20

TALON™ Low pH Elution Buffer

50 mM NaAc, pH 4,5
300 mM NaCl
0,01% Tween®-20

2. PROTOCOLS

2.1. Preparation of Sample Prior to Purification

There are many different ways of preparing a cell lysate containing expressed histidine-tagged proteins. It is important to note that the lysate can not contain EDTA (or other chelators), ionic detergents, DTT or DTE. A pH between 7 and 8 should be used.

Alternative lysis strategies for *E. coli* can be used, e.g.:

- Commercially available ready-made lysis buffers.
- TALON™ Binding and Washing Buffer with 1 % Triton® X-100.
- French press
- Sonication

Efficiency of lysis can be increased by the addition of Lysozyme.

To avoid a sticky pellet, the addition of DNaseI is recommended.

2.2. Manual Purification Protocol

Prepare your sample containing the histidine-tagged protein.

Thoroughly resuspend the Dynabeads TALON prior to use.

1. Transfer 50 µl (2 mg) Dynabeads TALON solution to a microcentrifuge tube. Place the tube on a magnet (Dynal MPC) until the beads have migrated to the side of the tube and the liquid is clear. Discard the supernatant. Equilibrate the beads with 700 ml TALON Binding and Washing Buffer and mix.
2. Again separate the beads from the buffer using a magnet and discard the buffer. Resuspend the beads in 100 µl TALON Binding and Washing Buffer.
3. Add your sample and adjust the total volume to 700 µl with TALON Binding and Washing Buffer. Incubate on a roller (or other continous mixing device) for 10 minutes at room temperature (or cold if the protein is unstable at room temperature).
4. Place the tube on a magnet until the beads have migrated to the side of the tube, then discard the supernatant.
5. Wash 4 times with 700 µl TALON Binding and Washing Buffer. Resuspend the beads thoroughly between each washing step. Place the tube on a magnet until the beads have migrated to the side of the tube and the liquid is clear, then discard the supernatant.

6a. If the protein is to be eluted, add 100 µl TALON Elution Buffer / TALON Low pH Elution Buffer. Leave the suspension on a roller for 5 minutes at room temperature (or cold if protein is unstable at room temperature). Collect the beads at the tube wall using a magnet and transfer the supernatant containing the eluted histidine-tagged protein to a clean tube.

Alternatively:

6b. If the protein is to remain bound to the beads, resuspend in TALON Binding and Washing Buffer or another buffer compatible with your downstream application.

2.3. Reuse of Dynabeads TALON

Dynabeads TALON may be reused. For regeneration protocols, please visit our website: www.dynalbiotech.com.

2.4. Automated Purification Protocols

Protein purification using Dynabeads TALON can easily be automated on a wide variety of platforms. Automation protocols are available at: www.dynalbiotech.com.

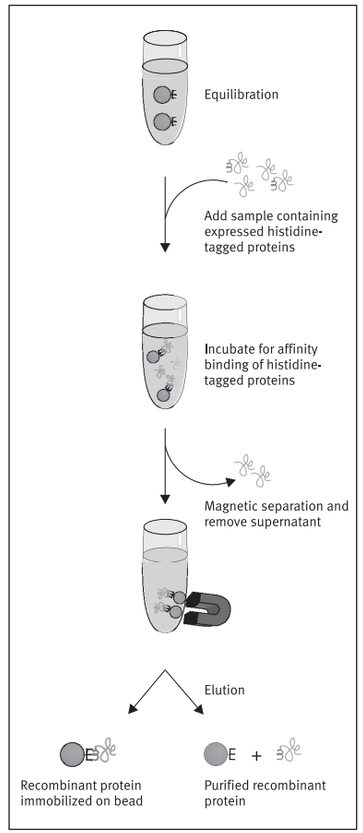

Fig. 2: Outline of the protocol for isolation of histidine-tagged recombinant proteins from a mixed starting sample using Dynabeads TALON.

3. TROUBLESHOOTING

Problem	Possible Cause	Suggested Solution
Isolated histidine-tagged protein is not pure enough.	Non-specific binding caused by endogenous proteins.	• Use low concentrations of imidazole (5-30 mM) in the washing and/or binding steps (not recommended when using the low affinity-tags). • Increase washing volumes. • Use a buffer with lower pH (pH 7.0) in the binding and/or washing steps. • Add one or two more washing steps, but note that the yield may decrease. • Make sure the beads are thoroughly resuspended during the washing steps. • Increase the salt concentration in the TALON Binding and Washing Buffer to 500 mM to inhibit non-specific ion interactions. • Add ethyleneglycol or glycerol to inhibit non-specific hydrophobic interactions in the binding and/or washing step. • Use a second purification step e.g. IEX, HIC or size exclusion.
	Too much DNA is present, resulting in a viscous pellet and thus decreasing the efficiency of washing.	• Add DNase to the lysate (e.g. 0.01 mg/ml DNaseI). • Sonicate the lysate to shear DNA.
The yield is too low.	Difficult to elute the protein.	• Use higher concentrations of imidazole (up to 500 mM) in the elution buffer. • Use a lowered pH in the elution buffer. • Alternatively, use an EDTA-buffer (e.g. 10-200 mM) for elution. Note that this will also release the Co^{2+}. • Increase the elution time.
	The protein is eluted in the washing buffer.	• Check the pH of the washing buffer, if necessary increase the pH slightly. • Avoid the use of imidazole in the washing buffer.
	Difficult to bind the protein.	• See suggestions below.
The protein does not bind.	The buffer composition in the binding step is not optimal.	• Check the pH and the compostion of the binding and/or washing buffers. Make sure the buffers do not contain chelating agents or ionic detergents. • Increase binding time.
	The tag is not exposed on the surface of the protein.	• Move the tag to the other end of the protein. • Change to a different histidine-tag. • Use denaturing conditions (e.g. 6M GuHCl, 8M Urea). If desired, refold the protein when bound to the beads.
	The protein is not adequatley expressed.	• Check your vector construct. • Add more inducing agent to the growing culture. • Change the expression conditions (e.g. 25ºC, shorter expression time, lower concentrations of inducing agent).
	The protein is insoluble.	• Use denaturing conditions (e.g. 6M GuHCl, 8M Urea). If desired, refold the protein when bound to the beads. • Change the expression conditions (e.g. 25ºC, shorter expression time, lower concentrations of inducing agent).
	The tag is degraded.	• See suggestions below
The protein is degraded.	Proteases have cleaved the protein.	• Add protease inhibitors to the lysate (e.g. PMSF or a protease inhibitor cocktail). • Work on ice. • Use "fresh" bacterial cultures. • Work quickly.
The beads aggregate.	Protein-protein interaction.	• Mix the beads more vigorously. • Increase the Tween-20 concentration (e.g. 0,05%) of the binding and/or washing buffers. • Add up to 20 mM ß-mercaptoethanol to the binding and/or washing buffers.

Problem	Possible Cause	Suggested Solution
The beads do not collect well at the magnet.	The solution is viscous. - OR The beads form aggregates because of protein-protein interaction	• Increase separation time (leave the tube on the magnet for 2-5 minutes). • Add DNAsel to the lysate (e.g. 0.01 mg/ml DNAsel. • Increase the Tween-20 concentration (e.g. 0,05%) of the binding and/or washing buffers. • Add up to 20 mM ß-mercaptoethanol to the binding and/or washing buffers.
The supernatant turns brown or grey.	The presence of DTT, DTE or high concentrations of ß-mercaptoethanol	• Completely remove the reducing agents e.g. with gel filtration or dialysis.
The supernatant turns pink.	Co^{2+}-leakage caused by EDTA, EGTA or other chelators.	• Completely remove the chelator e.g. with gel filtration or dialysis. (For protocols on regeneration/reuse, please wisit our website at: www.dynalbiotech.com.)
Non-compatible reagents.	• Dithiothreitol (DTT) or Dithioerythritol (DTE) • Ethylene diaminetetraacetic acid (EDTA), Ethylene glycol-bis(2-amino-ethyl)-tetraacetic acid (EGTA) or other chelators. A chelator can be used in the elution step, but then the Co^{2+} is released. • Ionic detergents.	• Completely remove the non compatible reagent e.g. with gel filtration or dialysis.
Problems related to the TALON™ chemistry	For more information about the BD TALON chemistry, please contact BD Biosciences Clontech	• See the 'BD TALON™ Metal Affinity Resins User Manual' that can be found on the web at: www.bdbiosciences.com

STORAGE & STABILITY

If stored unopened at 2-8°C upon delivery, Dynabeads TALON are stable until the expiration date stated on the label.

Note:

The vial should be stored upright to keep the Dynabeads TALON in liquid suspension, as drying of the Dynabeads will result in reduced performance. Do not freeze the product.

The Dynal MPCs should not be kept in close contact with magnetic tapes, computer discs or other magnetic storage systems, as these can be damaged by the strong magnetic field.

TRADEMARKS & PATENTS

The production and use of Dynabeads products are covered by several international patents and patent applications.

Dynal®, Dynabeads® and Dynal MPC® are registered trademarks of Dynal Biotech ASA, Oslo, Norway.

Technology used for Dynabeads® TALON™ is licensed from BD Biosciences Clontech, USA.

BD TALON™ is a trademark of Becton, Dickinson and Company, USA.

Tween® is a registered trademark of ICI Americas Inc, USA.

Triton® is a registered trademark of the Rohm and Hass Company, USA.

Dynal Biotech will not be responsible for violations or patent infringements which may occur with the use of our products.

WARNING & LIMITATIONS

Dynabeads TALON are for research use only. The product is not for use in human diagnostic or therapeutic procedures.

This product may not be repacked, reformulated or resold in any form without the written consent of Dynal Biotech ASA, Oslo, Norway.

This product contains 20 % EtOH as a preservative. Flammable liquid and vapour. Flash point 100°F (38°C). R-10 flammable.

Certificate of Analysis (CoA) is available upon requist. Material Safety Data Sheet (MSDS) is available at 'www.dynalbiotech.com'.

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. Dynal Biotech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Dynal Biotech's expense, of any products which shall be defective in manufacture, and which shall be returned to Dynal Biotech, transportation prepaid, or at Dynal Biotech's option, refund of the purchase price.

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

This warranty shall not apply to any products which shall have been altered outside Dynal Biotech, nor shall it apply to any products which have been subjected to misuse or mishandling. ALL OTHER WARRANTIES, EXPRESSED, IMPLIED OR STATUTORY, ARE HEREBY SPECIFICALLY EXCLUDED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Dynal Biotech's maximum liability is limited in all events to the price of the products sold by Dynal Biotech. IN NO EVENT SHALL DYNAL BIOTECH BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow limits on warranties, or on remedies for breach in certain transactions. In such states, the limits set forth above may not apply.

Dynal Biotech ASA will not be responsible for violations or patent infringements which may occur with the use of our products.

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