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 licensed 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 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".

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 tetradeutate 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 are able to occupy the two remaining coordination sites, resulting in protein binding.

Fig. 1: Dynabeads TALON with a bound alpha-chain 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 optimization may be required.

-**Dynabeads TALON** are supplied in 20% (v/v) sodium acetate buffer, pH 6.0.
-**Mixing device allowing rotation of tubes** (e.g. a roller or a Dynal® Sampler Mix) is provided.
-**Buffers**:
  - 50 mM NaAc, pH 4.5
  - 300 mM NaCl, 0.01% Tween®-20
  - 50 mM NaAc, pH 6.0
  - 300 mM NaCl, 0.01% Tween®-20
  - 300 mM NaCl
  - 300 mM NaCl
  - 300 mM NaCl, 50 mM Tris-HCl, pH 8.0

2. PROTOCOLS

2.1. Preparation of Sample Prior to Purification

There are many different ways of preparing a cell containing expressed histidine-tagged proteins. It is important to note that the lysate cannot contain EDTA (or other chelators), sonic 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.**
- **Induction® Binding** and **Washing Buffer with 1% Triton® X-100**.
- **Sonication**

Efficiency of lysis can be increased by the addition of lysozyme. To avoid a sticky pellet, the addition of DNase I is recommended.


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 M-1) until the beads have migrated to the side of the tube and the liquid is clear. Discard the supernatant. Equilibrate the beads with 700 µl of 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 of TALON Binding and Washing Buffer.
3. Add your sample and adjust the total volume to 700 µl with TALON Binding and Washing Buffer. Insolubilize the buffer with a magnet or (other continuous 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 bottom 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

Protocols for the purification of histidine-tagged recombinant proteins from mixed starting sample using Dynabeads TALON.

3. TROUBLESHOOTING

Problem | Possible Cause | Suggested Solution
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Isolated histidine-tagged protein is not pure enough | Non-specific binding caused by endogenous proteins. | • Add fresh to the lysate (e.g. 0.05% (v/v) DNase I).
| | | • Use fresh to the lysate (e.g. 0.01 M EDTA).
Too much DNA is present, resulting in a viscous pellet and thus decreasing the efficiency of washing. | • Add DNase I to the lysate (e.g. 0.05% (v/v) DNase I).
| | | • Use fresh to the lysate (e.g. 0.01 M EDTA).
Trouble with elution. | The yield is too low. | • Use higher concentration of imidazole (up to 500 mM) in the elution buffer.
| | | • Use a higher concentration of elution buffer.
| | | • Alternatively, use an EDTA buffer (e.g. 10-200 mM) for elution. Note that this will also release the Co²⁺.
| | | • Increase the elution time.
| | | • Use denaturing conditions (e.g. 6M Urea, 8M Urea). If desired, refold the protein when bound to the beads.
Protein is not adequately released. | The tag is not exposed on the surface of the bead. | • Use different elution conditions (e.g. 6M GuHCl, 8M Urea). If desired, refold the protein when bound to the beads.
| | | • Change the expression conditions (e.g. 25°C, 4°C) to increase the yield.
| | | • Change the expression conditions (e.g. 25°C, 4°C) to increase the yield.
| | | • Use denaturing conditions (e.g. 6M GuHCl, 8M Urea). If desired, refold the protein when bound to the beads.
Protein is not isolated. | The protein is not adequately released. | • Add more inducing agent to the growing culture.
| | | • Change the expression conditions (e.g. 25°C, 4°C) to increase the yield.
| | | • Use denaturing conditions (e.g. 6M GuHCl, 8M Urea). If desired, refold the protein when bound to the beads.
Proteins have cleaved the protein. | The tag is degraded. | • Use denaturing conditions (e.g. 6M GuHCl, 8M Urea).
| | | • Use denaturing conditions (e.g. 6M GuHCl, 8M Urea) if desired.
Proteins have cleaved the protein. | The protein is degraded. | • Add protease inhibitors to the lysate (e.g. PMSF or a protease inhibitor cocktail).
| | | • Work quickly.
Proteins have cleaved the protein. | The protein is degraded. | • Use fresh to the lysate.
| | | • Change the expression conditions (e.g. 25°C, 4°C) to increase the yield.
| | | • Use denaturing conditions (e.g. 6M GuHCl, 8M Urea). If desired, refold the protein when bound to the beads.
Proteins have cleaved the protein. | The beads aggregate. | • Mix the beads more vigorously.
| | | • Increase the Tween-20 concentration (e.g. 0.01%) of the lysate and/or washing buffers.
| | | • Add up to 20 mM M-mercaptoethanol to the binding and/or washing buffers.

**Problem**
The beads do not collect well at the magnet.

**Possible Cause**
The solution is viscous. OR The beads form aggregates because of protein-protein interaction

**Suggested Solution**
- Increase separation time (leave the tube on the magnet for 2-5 minutes).
- Add Dithiotreitol to the lysis (e.g. 0.05 M DTT)
- Increase the Tween-20 concentration (e.g. 0.05%) of the binding and/or washing buffers.
- Add up to 20 mM D-mannose to the binding and/or washing buffers.

**The supernatant turns brown or grey.**

**The presence of DTT, DTE or high concentrations of D-mannose is the cause.**

**Suggested Solution**
- Completely remove the reducing agents e.g. with gel filtration or dialysis.

**The supernatant turns pink.**

**CO2-leakage caused by EDTA, EDA or other chelators.**

**Suggested Solution**
- Completely remove the chelator e.g. with gel filtration or dialysis. (For protocols on regeneration/reuse, please visit our website at: www.dynalbiotech.com.)

**Non-compatible reagents.**

- Dithiothreitol (DTT) or Dithioerythritol (DTE)
- Thiourea or thio-glucosamine (ThG)
- Phosphate-buffered saline (PBS) or Tris buffers.

**Suggested Solution**
- Completely remove the non compatible reagent e.g. with gel filtration or dialysis.

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**STORAGE & STABILITY**

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

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**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.

Dynabeads®, Dynabeads® and DynaMPC® are registered trademarks of Dynabeads ASA, Oslo, Norway.

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

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

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

**TRITON®** is a registered trademark of the Rohm and Haas Company, USA.

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

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**WARNINGS & LIMITATIONS**

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

This product may not be repackaged, reformulated or resold in any form without the written consent of Dynal Biotech ASA, Oslo, Norway. This product contains 20 % EtOH as a preservative. It is flammable.

**Certificate of Analysis (CoA) is available upon request. 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 of Dynal Biotech’s product, or to 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, 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 limitations on warranties, or on remedies for breach in certain transactions. In such states, the limits set forth above may not apply.

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