Mag*Strep* Manual

Strep-Tactin coated magnetic beads for purification of _Strep_-tag fusion proteins

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IBA Headquarters
IBA GmbH
Rudolf-Wissell-Str. 28
D-37079 Göttingen
Germany
Tel: +49 (0) 551-50672-0
Fax: +49 (0) 551-50672-181
info@iba-go.com
www.iba-go.com

IBA US Distribution Center
10748 Indian Head Industrial Blvd.
St. Louis, MO 63132
Tel. 1-877-IBA-GmbH (1-877-422-4624)
Fax 1-888-531-6813
info@iba-go.com
www.iba-go.com

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Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and *Strep*-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use.

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1 Introduction

The *Strep*-tag® purification system is based on the highly selective binding of *Strep*-tag II fusion proteins to engineered streptavidin, called *Strep*-Tactin®. This technology allows one-step purification of recombinant proteins under physiological conditions, thus preserving their bioactivity.

Mag*Strep* (*Strep*-Tactin coated magnetic beads type 1) is a tool for the fast purification of *Strep*-tag proteins in batch format offering the possibility to work with small amounts of recombinant protein in solution*. Within minutes, the target molecules are bound specifically by *Strep*-Tactin®. Using a magnetic separator (Cat. No. 2-1602-000), the magnetic beads can be separated rapidly during the washing and elution steps, thereby rapidly enabling protein purification for further analysis.

* Please note that for expression rates below 1 mg/liter *E. coli* culture we highly recommend to use our *Strep*-Tactin® purification columns instead of performing batch purification.

2 Protocol

2.1 Preparation of a cleared lysate

Prepare the cleared lysate as described in the comprehensive manual "Expression and purification of proteins using *Strep*-tag and/or 6xHistidine-tag" available at "http://www.iba-go.com/download.html". Briefly, bacterial cells from 100 ml culture are sedimented by centrifugation and resuspended in 1 ml chilled Buffer W (100 mM Tris-Cl pH 8, 150 mM NaCl, 1 mM EDTA). Lyse cells via sonication or French Press under cooling and remove insoluble material by centrifuging at full speed in a microfuge for 15 min at 4 °C. Transfer the supernatant (cleared lysate) to a fresh tube and store on ice until purification. For longer storage the cleared lysate has to be stored at -20 °C. Then it has to be centrifuged again after thawing as new precipitates will have formed due to freeze/thawing.

The optimal way to prepare a cleared lysate for subsequent *Strep*-tag purification is to use the IBA-lyse reagent Kit (Cat. No.: 2-1017-050; 2-1017-250) for the lysis of bacterial cells.

Immediately prior to purification, pass the cleared lysate through a 0.45 µm filter.

2.2 Washing and equilibration of Mag*Strep* Beads

1. Determine how many magnetic beads are needed to purify the target protein (20 µl of the provided homogenous suspension correspond to 1 mg magnetic beads. Binding capacity of 1 mg magnetic beads corresponds to 90-110 pmol recombinant *Strep*-tag fusion protein). We recommend to work with 2 mg magnetic beads per purification (200 pmol of a 30 kDa protein correspond to 6 µg).
2. Place reaction tube with the required amount magnetic bead suspension in the magnetic separator
3. Remove supernatant
4. Wash beads three times with each 0.1 ml MagBuffer A per mg magnetic beads
5. Equilibrate beads in 0.05 ml MagBuffer W/I per mg magnetic beads
6. Place reaction tube with magnetic beads in magnetic separator
7. Remove supernatant - Magnetic beads are now ready to use.

2.3 Purification of recombinant *Strep*-tag® proteins using activated Mag*Strep* beads

1. When IBA-lyse has been used to prepare the cleared extract mix 60 µl of such extract with 40 µl MagBuffer W/I. In case of low expression rates 100 µl cleared IBA-lyse extract may be used without adding MagBuffer W/I.
   When the standard protocol from the manual "Expression and purification of proteins using *Strep*-tag and/or 6xHistidine-tag" has been used, mix 30 µl of the cleared extract with 70 µl MagBuffer W/I.
2. Mix with 2 mg of washed and equilibrated beads from section 2.2.
   In case of high expression rates (> 10 mg per liter culture) the amount of beads can be increased up to 5 mg to increase the yield.
3. Incubate 30 min at ambient temperature and shake occasionally (2-3x) bringing beads into suspension.
4. Place tube in a magnetic separator and remove supernatant.
5. Wash beads 3x with 100 µl MagBuffer W/I at each step.
   At each washing step: Add MagBuffer W/I, bring beads for 30 sec into suspension, place tube into magnetic separator, remove supernatant.
6. Elute recombinant *Strep*-tag fusion protein by adding 50 µl MagBuffer E to the beads. Incubate for 5 minutes under occasional shaking (2-3x) thereby bringing beads into suspension, place tube into magnetic separator and save supernatant.
7. Repeat step 6. using 30 µl MagBuffer E instead of 50 µl and save supernatant.
8. Pool supernatants from steps 6 and 7 and analyze protein content via SDS-PAGE and Coomassie or silver staining or via Western blotting.

3 Related products

Cat. No.	Product
2-1601-000	Mag*Strep* Kit
2-1601-002	Mag*Strep* Beads type 1; 2 ml
2-1601-005	Mag*Strep* Beads type 1; 5 ml
2-1602-000	Magnetic Separator for 24 rxn tubes
2-1017-050	IBA-lyse, Bacterial Lysis Buffer, suff. for 2.5 liter E. coli culture
2-1017-250	IBA-lyse, Bacterial Lysis Buffer, suff. for 12.5 liter E. coli culture