Protein purification using PureHT™ Strep-Tactin®XT plates with a liquid handling system

Automated Strep-tag® protein purification with 96 deep-well plates pre-filled with magnetic beads

1. Description

PureHT™ Strep-Tactin®XT plates contain dried MagStrep® Strep-Tactin®XT beads, which are ferrimagnetic spheres covered with 6% agarose and coupled with Strep-Tactin®XT. Strep-Tactin®XT specifically interacts with Strep-tag®II as well as Twin-Strep-tag® via its engineered biotin binding pocket and has a high affinity for both tags (nM range for Strep-tag®II and pM range for Twin-Strep-tag®). Due to the agarose surface and the high specificity of Strep-Tactin®XT, the magnetic beads show a very low non-specific protein binding. The binding capacity for target proteins is up to 0.85 nmol/µl beads (42.5 µg/µl of a 50 kDa protein).

This protocol describes the basic principle of automated protein purification using PureHT™ Strep-Tactin®XT plates, which contain 5 µl of dried MagStrep® Strep-Tactin®XT beads per well. It can be adapted to any liquid handling system of choice (e.g. Opentrons Flex, Tecan Fluent or Hamilton STAR).

2. Required material & recommendations

Example buffers/solutions		Cat. No.	Quantity
Wash buffer	Buffer W (10 x) (1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, pH 8)	2-1003-100	100 ml
Elution buffer	Buffer BXT (10 x) (1 M Tris-HCl, 1.5 M NaCl, 10 mM EDTA, 500 mM biotin, pH 8)	2-1042-025	25 ml
Regeneration buffer	100 mM NaOH (working concentration)	Not provided. Prepare freshly.	-

Please note that the wash & elution buffers are provided by Cube Biotech as 10-fold concentrated solutions. Prior to application, mix one part of 10-fold concentrated buffer with nine parts of deionized water. The composition of all purification buffers can be modified to suit the properties of the target protein. A list with compatible reagents is available at https://www.iba-lifesciences.com/download-area-protein.html. Please note that the magnetic beads are stable at pH 6-10.

Usually, protein purification and binding capacity of MagStrep® Strep-Tactin®XT beads are not influenced by free biotin for example in cell culture supernatants, but the co-purification of biotinylated proteins is possible. Biotinylated proteins are only present in the cell in very small amounts, but if a highly pure target protein is required, BioLock containing avidin can be applied. Avidin specifically masks biotinylated proteins without influencing the binding properties of the Twin-Strep-tag® or Strep-tag®II. The protocol for masking biotinylated proteins is provided at https://www.iba-lifesciences.com/download-area-protein.html.

The minimum recommended protein concentration for samples is 1 pmol/µl (50 ng/µl of a 50 kDa protein).

3. Protocol

3.1. Equilibration & washing of PureHT™ plate

3.1.1. Add **950 µl** Buffer W to each well.

3.1.2. Resuspend magnetic beads by pipetting up and down **50 times** at a speed of **≥ 160 µl/s.**

Optimal resuspension time depends on type of liquid handler used. To shorten resuspension time, increase flow rates or incubate PureHT™ plates with 950 µl Buffer W per well overnight at 4 °C.

3.1.3. Transfer the plate to a magnet and separate the magnetic beads by pipetting up and down **3 times** at a speed of ~**100 µl/s**.

Slowly pipetting up and down during magnet incubation steps may help ensure a faster and more complete separation of magnetic beads. The optimal pipetting speed can vary depending on the magnet used.

3.1.4. Remove the supernatant, then remove the plate from the magnet.

3.1.5. Add **950 µl** Buffer W to each well and wash the magnetic beads by pipetting up and down **10 times** at a speed of ~**160 µl/s**.

3.1.6. Transfer the plate to a magnet and separate the magnetic beads by pipetting up and down **3 times** at a speed of ~**100 µl/s**. Remove the supernatant.

3.1.7. Remove the plate from the magnet.

3.1.8. Repeat **steps 3.1.5.-3.1.7.** once.

3.1.9. The plate is now ready for protein purification (**3.2**).

3.2. Protein purification

Please note:

- During protein binding and elution, magnetic beads should stay in suspension. Optimal speeds for shaking or flow rates for pipetting up and down may vary depending on type of liquid handler used.
- During magnet incubation steps, slowly pipetting up and down may help ensure a faster and more complete separation of magnetic beads.

3.2.1. Add the sample containing the target protein to the wells of the PureHT™ plate prepared in **3.1.** Mix briefly to ensure proper resuspension of magnetic beads.

3.2.2. Incubate for **10 min** at room temperature by constantly shaking the plate at **700 rpm** or by pipetting up and down at a speed of ~**100 µl/s**.

3.2.3. Separate the magnetic beads by incubating the plate on a magnet and remove the supernatant.

3.2.4. Resuspend the magnetic beads with **500 µl** Buffer W. Incubate on a magnet and remove supernatant. Repeat this step twice.

3.2.5. Add **150-200 µl** 1x Buffer BXT to each well. Mix briefly to ensure proper resuspension of magnetic beads. Incubate for **10 min** at room temperature by constantly shaking the plate at **400 rpm** or by pipetting up and down at a speed of ~**40 µl/s**.

3.2.6. Separate the magnetic beads by incubating the plate on a magnet and transfer the eluted protein to a 96 deep-well plate.

The first elution step will yield the target protein at the highest concentration. To maximize yield, repeat the elution step once and combine elution fractions.

3.2.7. Separate remaining magnetic beads by incubating the plate on a magnet and transfer the target protein to a plate of your choice for further downstream applications.

3.3. Regeneration of magnetic beads (optional)

Please note:

- Regeneration in batch may be incomplete if done incorrectly.
- A decrease in yield following regeneration may be attributed to magnetic bead loss associated with certain liquid handling instruments.
- During magnet incubation steps, slowly pipetting up and down may help ensure a faster and more complete separation of magnetic beads.

3.3.1. Freshly prepare the regeneration buffer (**100 mM NaOH**) before use.

3.3.2. Add **500 µl** of regeneration buffer to each well containing the magnetic beads. Mix briefly to ensure proper resuspension of magnetic beads.

3.3.3. Incubate for **2 min** at room temperature. Separate the magnetic beads by incubating the plate on a magnet and remove the supernatant.

3.3.4. Add **500 µl** Buffer W to each well to equilibrate the magnetic beads. Separate the magnetic beads by incubating the plate on a magnet and remove the supernatant. Repeat this step twice.

3.3.5. Add **100 µl** Buffer W to each well. The plate containing the magnetic beads can be stored **at 2-8 °C** or used again from **3.1.3**.