Protein purification using PureHT™ Strep-Tactin®XT plates with a KingFisher Flex instrument

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 how to perform automated protein purification using PureHT™ Strep-Tactin®XT plates, which contain 5 µl of dried MagStrep® Strep-Tactin®XT beads per well, with a KingFisher Flex instrument.

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. Protein purification

Download the program for protein purification with KingFisher Flex here.

3.1.1. Prepare the 96 deep-well plates for the KingFisher system as follows (volumes/well):

1. Tip comb plate	**6.** Wash Plate with **500 µl** Buffer W
2. PureHT™ plate with **950 µl** Buffer W	**7.** Wash Plate with **500 µl** Buffer W
3. Equilibration Plate with **950 µl** Buffer W	**8.** Wash Plate with **500 µl** Buffer W
4. Equilibration Plate with **950 µl** Buffer W	**9.** Elution Plate with **50-100 µl** Buffer BXT
5. Plate with the target protein	

Load plates into the instrument according to the protocol requests displayed on the KingFisher Flex screen, placing each plate in the same orientation. Confirm each action by pressing **Start**.

3.1.2. Resuspend the magnetic beads in **plate 2** at "fast" speed for **20 min**.

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

3.1.3. Transfer the magnetic beads to **plate 3** and mix at least **2 min** at "medium" speed. Repeat this step inside **plate 4**.

If you want to continue protein purification at a later time point, transfer magnetic beads to a plate containing 100 µl Buffer W and store the plate at 2-8 °C until further use.

3.1.4. Transfer the magnetic beads to **plate 5** containing the target protein and incubate at **room temperature** for **10 min** at "medium" speed.

3.1.5. Wash the beads for **2 min** in **plate 6** at "medium" speed. Repeat this step with **plate 7** and **plate 8**.

Depending on protein, increasing the washing time to up to 10 min can improve purity.

3.1.6. Transfer the magnetic beads to **plate 9** and incubate by mixing at "medium" speed for **10 min**.

3.1.7. Transfer the magnetic beads to **plate 2**. **Plate 9** contains the target protein.

3.2. Regeneration of magnetic beads (optional)

Please note that the regeneration in batch may be incomplete if done incorrectly. The magnetic beads can be regenerated 3-5x (depends on KingFisher instrument) without a decrease in yield.

Download the program for magnetic bead regeneration with KingFisher Flex here.

3.2.1. Prepare the 96 deep-well plates for the KingFisher system as follows (volumes/well). Freshly prepare the regeneration buffer (**100 mM NaOH**) before use.

1. Tip comb plate	**5.** Wash Plate with **500 µl** Buffer W
2. Plate with magnetic beads	**6.** Wash Plate with **500 µl** Buffer W
3. Regeneration Plate with **500 µl** 100 mM NaOH	**7.** Storage Plate with **100 µl** Buffer W
4. Wash Plate with **500 µl** Buffer W	

Load plates into the instrument according to the protocol requests displayed on the KingFisher Flex screen, placing each plate in the same orientation. Confirm each action by pressing **Start**.

3.2.2. Transfer the magnetic beads to **plate 3** and mix for **30 s** at "medium" speed and **2 min** at "slow" speed.

3.2.3. Transfer the magnetic beads to **plate 4** and mix for **30 s** at "medium" speed and **2 min** at "slow" speed. Repeat this step inside **plate 5 and 6**.

3.2.4. Transfer the magnetic beads to **plate 7**.

3.2.5. The plate containing the magnetic beads can be stored **at 2-8 °C** or used again from **step 3.1.4.**