Protein purification using PureHT™ Ni-INDIGO plates with a KingFisher Flex instrument

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

1. Description

PureHT™ Ni-INDIGO plates contain dried PureCube Ni-INDIGO MagBeads, which are ferrimagnetic spheres covered with 6% agarose and coupled with the specially engineered INDIGO ligand. Due to stable immobilization of nickel ions (Ni) to INDIGO, the MagBeads tolerate 20 mM EDTA and 20 mM DTT. Due to the agarose surface, the MagBeads have a high binding capacity and show very low non-specific protein binding. The binding capacity for target proteins is up to 100 mg/ml of a 27 kDa protein.

This protocol describes how to perform automated protein purification using PureHT™ Ni-INDIGO plates, which contain 2.5 µl of dried PureCube Ni-INDIGO MagBeads beads per well, with a KingFisher Flex instrument.

2. Required material & recommendations

Example buffers/solutions		Quantity
Binding Buffer	50 mM NaH_2PO_4, 300 mM NaCl, 10 mM Imidazole, pH 8.0	200 ml
Wash Buffer	50 mM NaH_2PO_4, 300 mM NaCl, 20 mM Imidazole, pH 8.0	300 ml
Elution Buffer	50 mM NaH_2PO_4, 300 mM NaCl, 250 mM Imidazole, pH 8.0	10-20 ml

Please note that Ni-INDIGO MagBeads can tolerate up to 20 mM DTT and 20 mM EDTA. Depending on the protein properties, adding those to the buffers may increase protein yield. Screening of buffer composition and pH for optimal target protein conditions can be useful. Optimizing the imidazole concentration can increase protein yield and purity.

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** Washing Buffer
2. PureHT™ plate with **950 µl** Binding Buffer	**7.** Wash Plate with **500 µl** Washing Buffer
3. Equilibration Plate with **950 µl** Binding Buffer	**8.** Wash Plate with **500 µl** Washing Buffer
4. Equilibration Plate with **950 µl** Binding Buffer	**9.** Elution Plate with **50-100 µl** Elution Buffer
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 Binding Buffer 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 binding buffer 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 **30 min** at "medium" speed.

To maximize yield, increase incubation time to 1 h.

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 1**. **Plate 9** contains the target protein.