Protein purification using PureHT™ Ni-INDIGO plates with a liquid handling system

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 the basic principle of automated protein purification using PureHT™ Ni-INDIGO plates, which contain 2.5 µl of dried PureCube Ni-INDIGO MagBeads 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		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. Equilibration & washing of PureHT™ plate

3.1.1. Add **950 µl** Binding Buffer 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 Binding Buffer 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** Binding Buffer 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 **30 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**.

Increasing the incubation time to 1 h may maximize the protein yield.

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** Wash Buffer. Incubate on a magnet and remove supernatant. Repeat this step twice.

3.2.5. Add **150-200 µl** Elution Buffer 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.