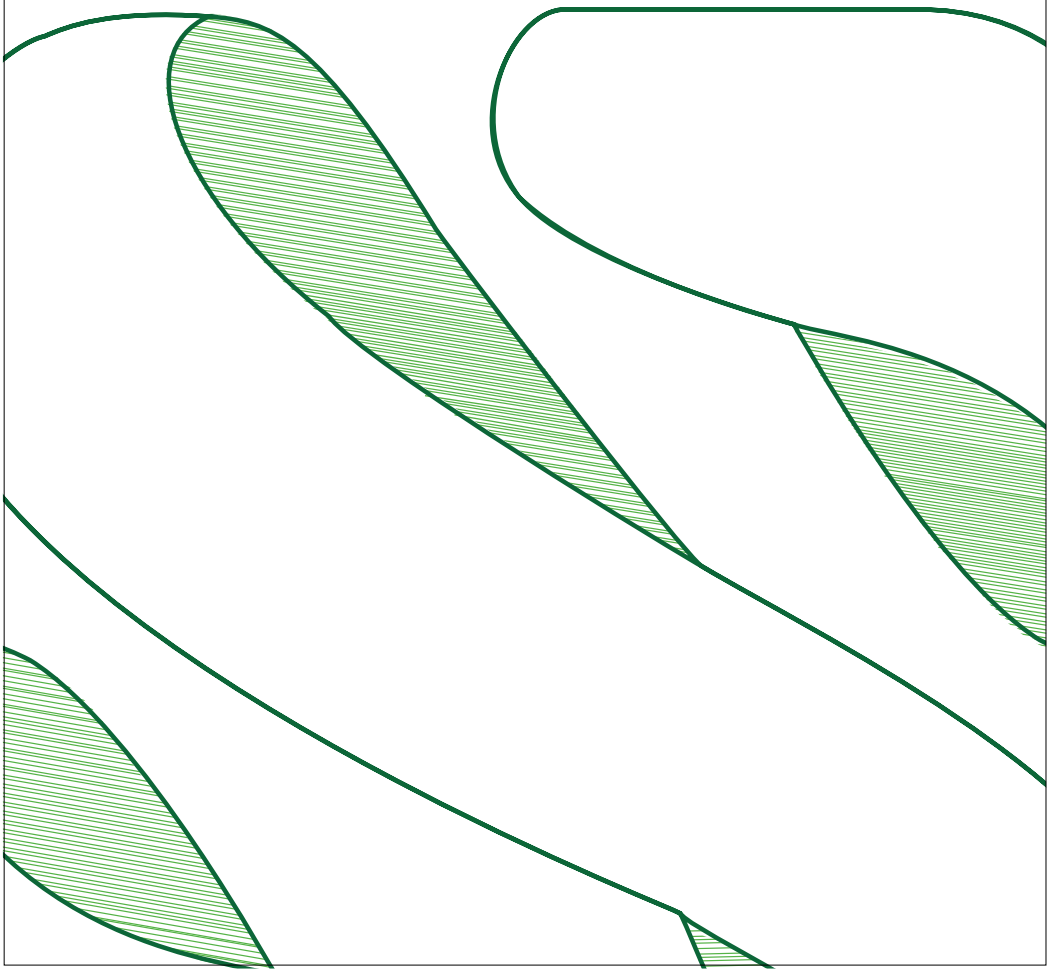
HisCube Ni-INDIGO

His-tag Protein Purification MIDI Kit



HisCube - Ni-INDIGO

His-tag Protein Purification MIDI Kit

Contents	2 x 50 mL Binding Buffer 2 x 50 mL Wash Buffer 1 x 50 mL Elution Buffer 10 mL Ni-INDIGO-Resin 500 mM EDTA (add 0.866 mL ddH_2O) 500 mM DTT (add 1.016 mL ddH_2O) 5 x MIDI centrifuge columns
Required equipment	Pipettes & matching tips Table centrifuge 1.5 mL tubes Syringe + Filter for sterilization End-over-end shaker 50 mL falcon Tubes
Storage temperature	4 °Celsius
Online Version	https://cube-biotech.com/HisCube-Ni-INDIGO-His-tag-Protein-purification-MIDI-protocol
Support	https://cube-biotech.com/ Live Chat Support contact@cube-biotech.com

1. Introduction

Thank you for choosing our HisCube - Kit, the superior alternative to traditional Ni-NTA based His-tag protein purifications.
This kit aims for the upmost purity and highest possible protein yield that IMAC based His-tag purifications can achieve.

The INDIGO-Ligand was developed by Cube Biotech and has proven numerous times to be the best option to reach maximum purity & protein yield simultaneously.

The PureCube Ni-INDIGO resin consist of 6% cross-linked agarose beads with an average diameter of 100 µm. The resin has a 50% concentration and a protein binding capacity of 100 mg / mL. The provided spin-columns are suited for laboratory centrifuges.

1.1 What sets this kit apart from other His-tag purification kits?

The novel feature that sets this kit apart from traditional His-tag kits is our in house developed **INDIGO ligand.**

The key advantage of the INDIGO ligand compared to other options like NTA or IDA is that it is capable to **tolerate 20 mM EDTA and 20 mM DTT** as part of the purification buffers.

In comparison: NTA tolerates 10 mM DTT and 1 mM EDTA. The advantages of DTT and EDTA are listed later on.

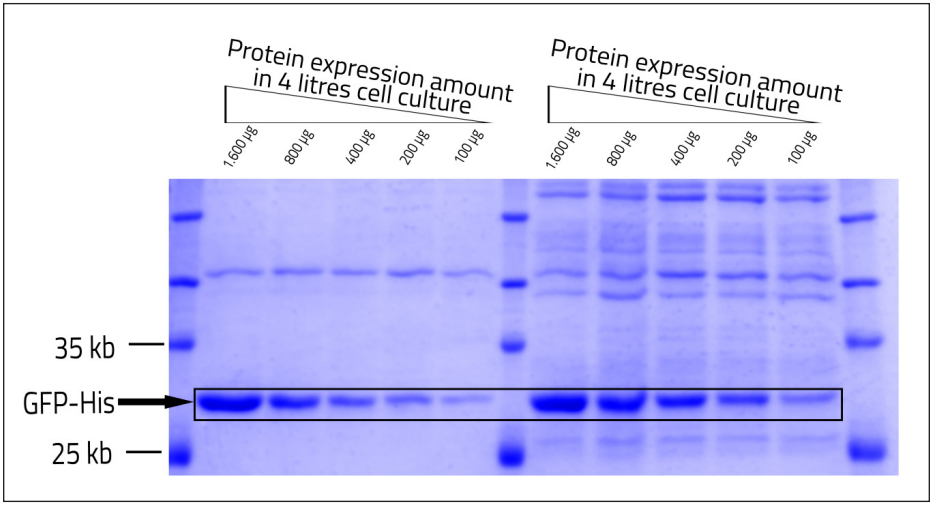


Figure 1: *Ni-INDIGO (left) resin compared to Ni-NTA from another competitor (right). This test compared the protein yields & purity of both resins using controlled amounts of His-tagged GFP in the corresponding cell lysates.*

2. Contents

10 mL MIDI centrifuge columns: These 10 mL columns are disposable polypropylene devices with polyethylene filter discs designed for use in gravity-flow or centrifugation-based applications involving chromatographic resins. Centrifuge columns provide a rapid and inexpensive alternative to typical gravity- or pressure-based chromatographic separations.
10 mL columns are designed for 50 mL falcon tubes.

Buffers: The HisCube Ni-INDIGO Purification Kit contains three different buffers.
Each buffer is optimized for our Ni-INDIGO beads. Note that the Elution Buffer contains 250 mM Imidazole. Please read the section "Protein quantification" regarding the effect of Imidazole on protein quantification and how to overcome it.

2.1 Why are EDTA & DTT included in this kit?

EDTA & DTT have great advantages in a lot of buffers involved in protein purification, but unfortunately ligands like NTA or IDA get stripped by them and therefore loose their functionality.

DTT involving buffers can prevent protein aggregates since it dissolves disulfid bridges between proteins.

Note: DTT becomes instable after being dissolved in water. Only add it to the buffers directly prior to its use. Freeze the remaining stock at -20°C.

EDTA can chelate cations that are co-factors of some proteases. Therefore it can function as a protease inhibitor. This results in higher protein yields. EDTA is mostly effective as part of the Binding Buffer.

Both EDTA & DTT come lyophilized. Simply add the given **volumes of ddH_2O** to the powders to get a **500 mM stock solution** of each substance. Both their final concentration in the buffers should be around **1 - 5 mM.**

2.2 The Ni-INDIGO resin

The INDIGO ligand is a novel development of Cube Biotech and is the solution to a lot of problems that plague His-tag protein purifications.
As Figure 1 shows, His-tag purifications using the INDIGO ligand are much purer than traditional Ni-NTA.

Ni-INDIGO resin beads are composed of 6% cross-linked agarose with an average bead diameter of 100 µm. The resin can be used in MIDI sized drip/spin coloumns as demonstrated in this kit.

It can bind up to **100 mg protein per mL** resin used**.**

3. Procedure / Protocol

Every protein purification assay starts with the extraction of the protein of interest from the expression cell.

This procedure is dependent on the used expression system. As already stated our Ni-INDIGO resin is compatible with protein lysates from *E.coli*, mammalian cell lines, insect cell and yeast to name a few.

Possible mechanical disruption methods are freeze/thaw cycles, sonication, French press or glass bead lysis (yeast). Please refer to your own lysis protocol.

For small scale purification we recommend sonication.

3.1 Purification steps

In before: Later on a flow-through fraction and wash-fractions are mentioned. Make sure to keep them!

1. Shake the Ni-INDIGO resin flask well to mix the settled beads with the storage buffer. **If** you are using EDTA or DTT add them now to the Binding, Wash and Elution Buffers.

2. Place the MIDI centrifuge column in a conventional 50 mL (falcon) tube.

3. Pipet 2 mL of the 50% Ni-INDIGO slurry into the batch spin column. Centrifuge the resin at 3000 x g for 20 seconds. Remove the flow-through.

Note: This step 3 is critical to ensure that all ethanol is removed from the resin to avoid interference with the membrane technology.

INDIGO-Ni storage buffer contains 20% Ethanol.

4. Equilibrate the MIDI centrifuge column with 5 ml binding buffer by centrifuging the spin column at 3000 x g for 20 seconds. Remove the flow-through.

5. Repeat step number 4 once to remove any residual ethanol.

6. **Immediately before loading** re-filter the sample through a 0.2 µm filter (e.g. syringe filter) to remove any solid material that might clog the column.

Note: It is critical to perform this step immediately before loading the sample on the column to ensure optimal performance.

7. Load the required volume of filtered sample. The maximum sample volume is 10 mL. Close the lid and vortex for 15 seconds to mix the sample and the resin.

8. *Alternative A:* Repeat the vortexing every 15 min for 1 hour. In some circumstances, more than 1 hour batch incubation may be required. Repeat the vortexing every 30 min – 1 hour.

   or

8. *Alternative B:* Incubate on an end-over-end shaker at room temperature for 30 min, or at 4°C for 1 h, depending on the temperature stability of the protein.

9. Centrifuge the lysate at 3000 x g for 20 seconds. Save the flow-through. This flow-through is called **flow-through fraction.**

10. Load the spin column barrel with up to 3 mL of Wash Buffer. Vortex or invert the closed tube a couple of times. This spin at 3000 x g for 20 seconds. It is best to save the flow-through. This flow-through is called **wash fraction.**

Note: The flow-through contains the wash fractions. Keep aliquots of the individual wash fractions for subsequent SDS-PAGE analysis.

11. Repeat the wash step for at least two times to ensure removal of unspecifically bound protein. If applicable, check the samples for protein content using a UV-spectrophotometer. Absorbance at 280 nm should be < 0.1.

12. Elute the target protein by adding 0.6 - 6 mL Elution Buffer and centrifuging at 3000 x g for 20 seconds. If necessary, repeat the elution step up to 5 times.

Note: This 12-fold range of Elution Buffer volumes is meant to allow the adjustment of the final volume to the protein expression level. Low expressed proteins should not be eluted in a big volume.

13. Save each eluate fraction in a separate new matching tube. Determine the protein concentration by measuring absorbance at 260 - 280 nm.

Optional: Use a fresh tube for the elution step to avoid contamination with the previous wash fractions.

3.2 Quantification

It is important to remember that the Elution Buffer contains 250 mM Imidazole. This substance has its own absorbance at 280 nm, similary to proteins. Therefore it must be removed in before. For this purpose we recommend

to perform a dialysis of your eluated protein. Now you can measure its absorbance at 280 nm.

4. Troubleshooting

In before: If your problem can not be solved by this troubleshooting guide, feel free to state your problem directly to:
contact@cube-biotech.com

Protein Yield: Problems with the protein yield can be numerous, follow this checklist to avoid the most commonly made ones.

- **Expression:** Always confirm that your protein is indeed expressed. Check your lysate e.g. through a Coomassie stain, to confirm the presence of your protein of interest.
- **Expression II:** Afterwards you should perform a Western Blot with e.g. a PentaHis antibody to exclude the possibility of a false-positive result of the Coomassie stain.

- **Tag Accessibility:** Occasionally the 3D-structure of a protein can mask the His-tag and therefore hinder the Ni-INDIGO bead's access to the tag. There are two ways to prevent this: First, change the terminus of the protein where the His-tag is attached to. Second, add a spacer sequence between the protein's terminus and the His-tag.

Protein Purity: Add additional washing steps, by additional repeats of **step 10** or slightly increase the imidazole concentration of the Wash Buffer to get rid of unwanted molecules with less affinity to the beads.

Column flow: Most of the time the column flow is hindered by cell debris that is left over in the cell lysate. Remember to follow **step number 6** of the protocol to prevent this.

Protein aggregates: Sometimes proteins can aggregate in a lysate. This prevents proper binding to the Ni-INDIGO beads. To counter this, add around 1 - 5 mM DTT to all buffers.

Active proteases: Sometimes the protein of interest is degraded by proteases that can be found inside the cell lysate. These need to be inhibited.
As proteases usually work with cations as co-factors, EDTA will chelate these cations and thus inactivate the proteases.
If this is the case we recommend to add 1 - 5 mM EDTA to at least the Binding Buffer.

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