Automated Ni-NTA bead-based protein purification on the OT-2 liquid handler

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ABSTRACT

An automated protocol was developed to perform magnetic bead-based immobilized metal affinity chromatography (IMAC) on the Opentrons OT-2 liquid handler. We tested the protocol using the Pierce™ Ni-NTA Magnetic Agarose Beads (Thermo Fisher Scientific, Waltham, MA, USA) for extracting the protein of interest (His-tagged GAPDH) on the OT-2 using samples prepared by diluting recombinant GAPDH protein in lysis buffer, HEp-2 cell lysates, and bacterial cells.

KEY FINDINGS

The OT-2 demonstrated competency for purifying recombinant GAPDH protein using Ni-NTA magnetic beads.

- Protein analysis confirms that the target protein was extracted with desirable consistency and specificity.
- In a higher-throughput setting, this protocol can handle up to 96 samples with minimal hands-on time.

INTRODUCTION

The aim of the protein purification is to obtain stable and active proteins in high purity for downstream analytical or research use or for therapeutic applications. Immobilized metal affinity chromatography (IMAC) utilizes metal ions to extract a recombinant protein with a genetically engineered tag, usually a peptide that can chelate metal ions. Nickel-nitrilotriacetic acid (Ni-NTA) coupled with agarose resin or magnetic beads is a popular IMAC tool to purify polyhistidine (His)-tagged protein. Since His residue can chelate nickel ions (Ni^{2+}), His-tagged proteins exhibit a strong affinity to a carrier on which Ni^{2+} is immobilized. An automated protein purification protocol was performed on the OT-2 liquid handler to extract His-tagged recombinant glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using Pierce Ni-NTA Magnetic Agarose Beads.

MATERIAL AND METHODS

Overview of the Ni-NTA bead-based protein purification workflow on the Opentrons OT-2 platform
The IMAC protocol was divided into several sections: Part 1 (sample/bead mixture preparation), target capture (not on the OT-2 deck), Part 2 (washing and elution), as summarized in **Figure 1**. Both Part 1 and 2 were performed on the OT-2. To process 96 samples, Part 1 has a runtime of 57 min followed by a 30 min target capture on a shaker. Part 2 takes 78 min to complete.

Schematic of OT-2 deck layout for Ni-NTA protocol
Part 1 and Part 2 of the protocol were performed on the OT-2 platform with a GEN2 Magnetic Module for separating the magnetic beads from the solution **(Figure 2)**.

Samples were processed by mixing 500 µL human His-tagged GAPDH solution with 12.5 µL settled beads and then agitated at 800 rpm for 30 minutes at room temperature. After 2 washing steps, the target protein was eluted in a 250 µL elution buffer on a shaker for 10 minutes at 800 rpm and room temperature. 20 µL eluate was subjected to SDS-PAGE and Western Blot analysis using the monoclonal anti-GAPDH antibody (Thermo Fisher Scientific, Waltham, MA, USA) and a fluorescent-dye conjugated secondary antibody (LI-COR Biosciences, Lincoln, NE, USA).

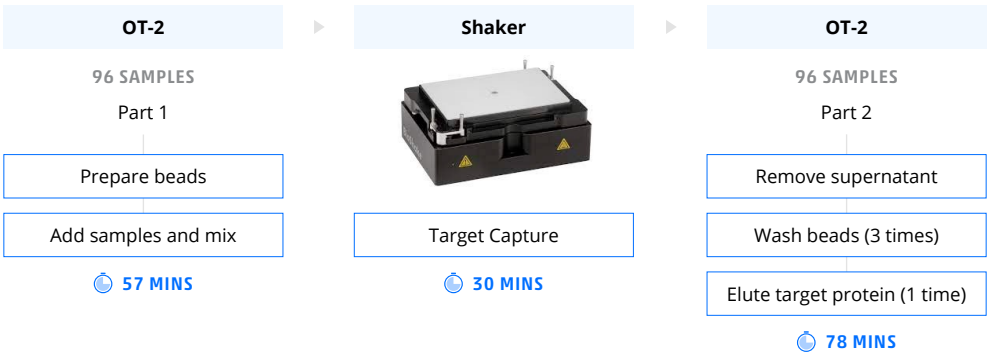


Figure 1: Overview of the Ni-NTA bead-based protein purification workflow on the Opentrons OT-2 platform.

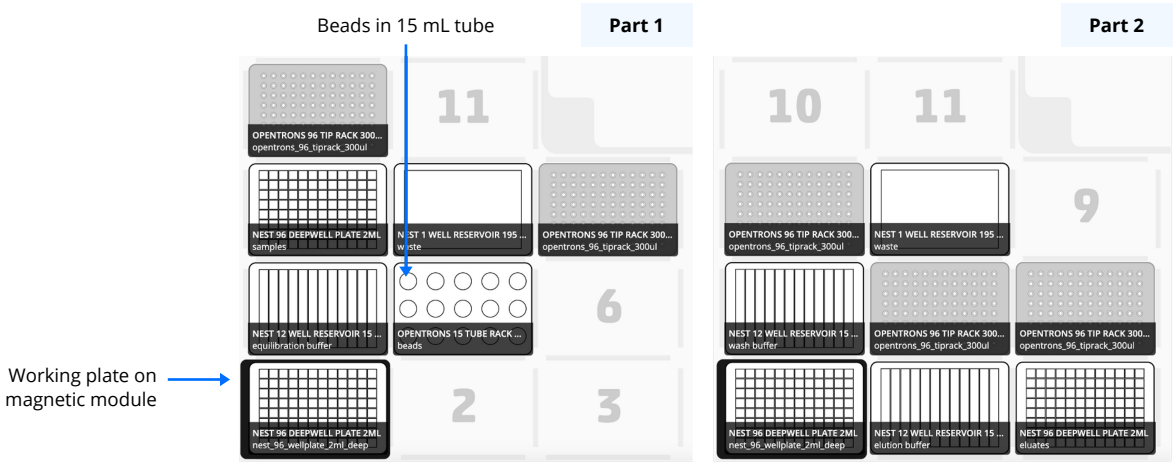


Figure 2: Schematic of OT-2 deck layout for Ni-NTA protocol.

RESULTS

GAPDH was extracted with desirable consistency of sample handling in quintuplicate (CV = 6%, measured by analysis of the signals emitted by protein bands as shown from Western blot analysis) **(Figure 3)**. Some extracts were quantified by using Qubit™ protein assay (Thermo Fisher Scientific, Waltham, MA, USA) to demonstrate the high protein yield **(Figure 4A)**. In addition, the protein purification processed by the OT-2 was not disruptive as the extracted protein retained its enzymatic activity, measured by GAPDH activity assay (Sigma-Aldrich, St. Louis, MO, USA) **(Figure 4B)**.

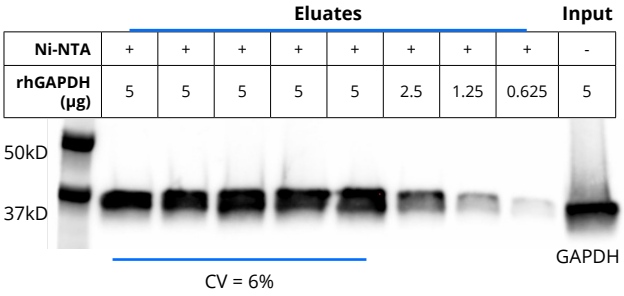
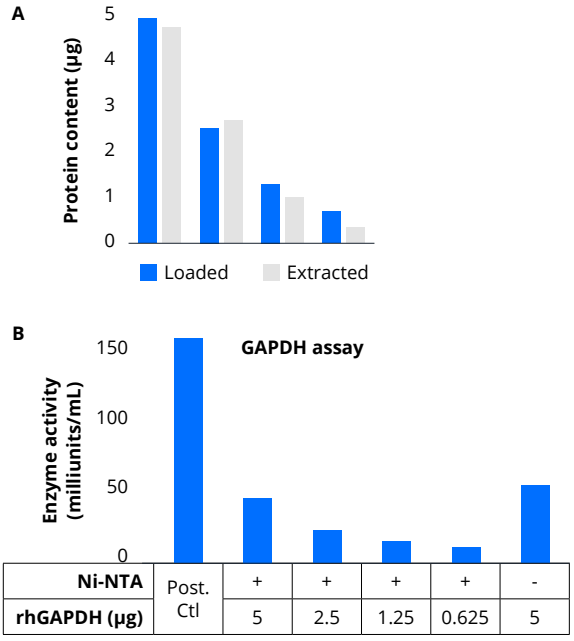


Figure 3: His-tagged GAPDH was extracted with desirable consistency.

Ni-NTA-mediated pull-down is particularly useful to isolate ectopically expressed His-tagged proteins in cells or tissue samples. The protocol described previously was performed to purify His-tagged GAPDH in 200 µL HEp-2 cell lysate. The results again demonstrated that His-GAPDH was successfully extracted, and compared to the binding of untagged protein, the affinity of Ni-NTA beads was highly specific **(Figure 5)**.

Recombinant protein expression in *Escherichia coli* is a common approach for larger scale protein production. Cell lysate was prepared by lyzing His-tagged GAPDH expressing bacterial cells with or without IPTG induction. The results further confirmed that the protocol successfully performed His-tag assisted protein purification using Ni-NTA magnetic beads **(Figure 6)**.

In this study, the protocol was set to process 96 samples, the results presented in this study showed 8 samples loaded in the last column of a 96-well sample plate. The runtimes of various sample sizes were also estimated **(Figure 7)**.



Ni-NTA	Post. Ctl	+	+	+	+	-
rhGAPDH (µg)		5	2.5	1.25	0.625	5

Figure 4: Qubit protein assay demonstrated a high protein yield, and extracted protein retained its enzymatic activity.

	Eluates								Input
Ni-NTA	+	+	+	+	+	+	+	+	-
rhGAPDH untagged (µg)	5	-	-	-	-	-	-	-	5
rhGAPDH His-tagged (µg)	-	5	2.5	1.25	0.625	0.313	0.156	0.078	-

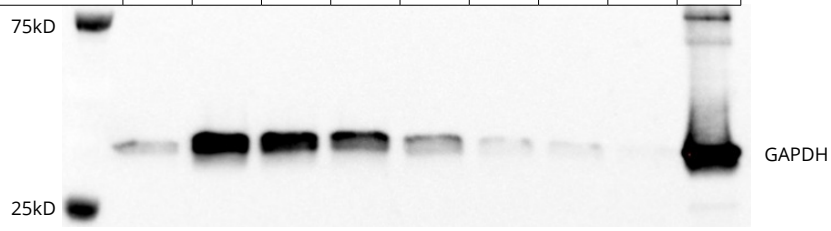


Figure 5: His-GAPDH was successfully extracted from HEp-2 cell lysate with high specificity.

	Eluates								Input
Ni-NTA	+	+	+	+	+	+	+	+	-
BL21 (IPTG+)	+	+	+	+	-	-	-	-	+
BL21 (IPTG-)	-	-	-	-	+	+	+	-	-
rhGAPDH His-tagged (µg)	-	-	-	-	-	-	-	5	-

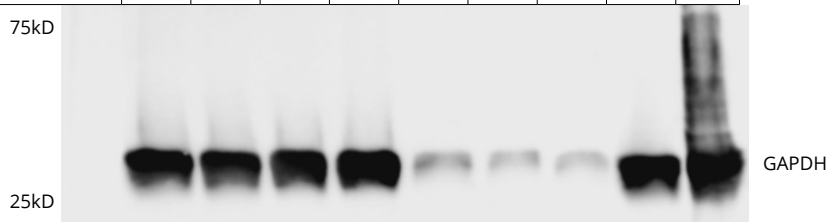
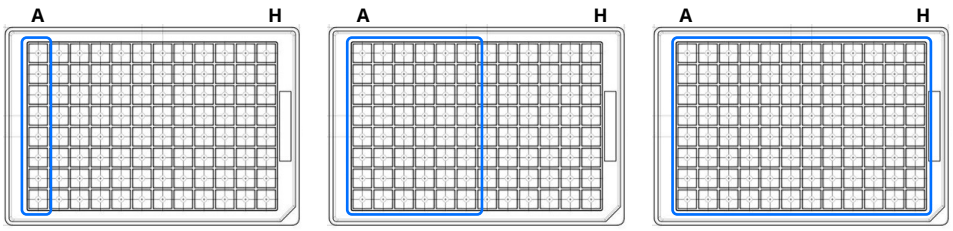


Figure 6: His-GAPDH was expressed in bacterial culture and successfully extracted.



	8 Samples		48 Samples		96 Samples	
Protocol	Runtime (min)	Tip boxes	Runtime (min)	Tip boxes	Runtime (min)	Tip boxes
Part 1	11	1	31	1	57	2
Incubation	30	-	30	-	30	-
Part 2 (w/ elution x1)	28	3	54	3	78	3
Sum	69	4	115	4	165	5
Part 1	11	1	31	1	57	2
Incubation	30	-	30	-	30	-
Part 2 (w/ elution x2)	41	3	81	3	104	3
Sum	82	4	142	4	191	5

Figure 7: Runtimes and tip boxes required (one elution vs. two elutions).

CONCLUSION

A robust automated workflow solution for magnetic bead-based purification of His-tagged recombinant GAPDH protein was performed on the OT-2. The OT-2 can process samples in medium- to high-throughput settings, reducing hands-on-time while providing desirable reproducibility and specificity.