

TheOnyx

Automated Purification of 6xHis-tagged Proteins

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Overview

High throughput screening of recombinant proteins has become a widely used application in recent years. Cell growth and protein production can be performed overnight with very little hands-on time by incubating cell cultures in a heater-shaker. However, a process bottleneck often exists when performing the downstream steps of cell lysis and protein purification. Here we describe the complete automation of these steps using a magnetic bead protocol for the purification of 6xHis-tagged recombinant proteins induced in *E.coli*. The system is based on the selective affinity of nickel-nitrilotriacetic acid (Ni-NTA) for proteins or peptides with six consecutive histidine residues.

Integrated modules needed for this application

- Microtiter plate position
- Reagent rack
- Reagent rack with stirrer for magnetic beads (Figure 1)
- Magnetic separator
- Integrated microplate shaker

Method

Cell growth and storage

For the expression of recombinant proteins *E.coli* strain BL21 DE3 (Invitrogen) was used in conjunction with the pET expression vector system (Novagen). Cells were grown to $OD_{600} = 0.5$ (37°C , 300 rpm) induced with the appropriated inducer, and grown for an additional 3 hours.

The cells were then harvested by centrifugation. Cell pellets were stored frozen at -20°C , then brought to room temperature before purification.

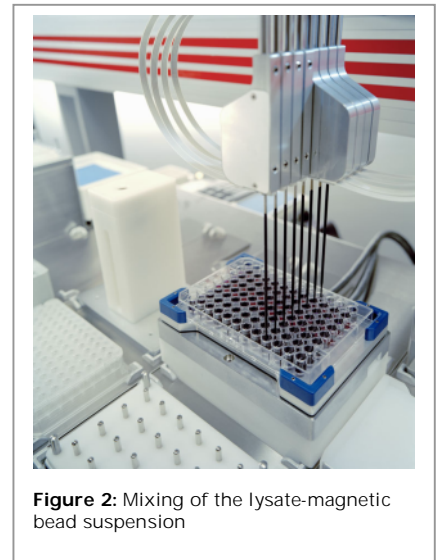


Figure 1: Reagent rack with stirrer for magnetic bead solution

Purification Procedure

All liquid handling and plate movement steps were performed by TheOnyx.

1. Deep well plates containing cell pellets were placed on a cooled position on TheOnyx platform together with lysis buffer (BugBuster HT protein extraction reagent, Novagen Cat. No. 70922-4), 5% (v/v) NiNTA magnetic agarose bead suspension (Qiagen, Cat. No. 1014552), phosphate washing buffer (20 mM imidazole) and elution buffer (250 mM imidazole).
2. 200 μ l of lysis buffer was distributed into each well, and the cells resuspended by repeated aspiration & dispensation. The plate was then moved to the integrated plate shaker, and cell lysis performed by shaking at 1000 rpm for 10 minutes.
3. Lysates were transferred to a fresh 96 flat bottom plate into which magnetic bead suspension (50 μ l per well) had been distributed. The resulting lysate-magnetic bead suspensions were shaken at 1000 rpm for 30 minutes (**Figure 2**).
4. The resulting protein-bead complexes were pelleted by transferring the plate onto the integrated magnetic separator. Lysate supernatants were discarded by carefully pipetting from the bottom of each well.
5. Pelleted protein-bead complexes were resuspended and washed 3 times by sequentially adding 200 μ l, 150 μ l and 100 μ l of phosphate buffer (20 mM imidazole). Shaking at 1000 rpm for 10 minutes. After each washing step the bead complexes were pelleted and the supernatants discarded as before.
6. Proteins were eluted from beads as protein-metal complexes by the addition of 50 μ l 250 mM imidazole (imidazole selectively binds to the beads so that the proteins are released). The beads were pelleted once more by transferring the plate to the magnetic separator, and the purified proteins left behind in the supernatant transferred to a fresh result plate.



Results

From an initial cell culture volume of 1 ml the proteins were eluted from the beads in a volume of 50 μ l. The resulting concentration of purified 6xHis-tagged protein (53 KD) was in the range 0.15 – 0.30 μ g/ μ l. **Figure 3** illustrates 48 randomly chosen samples from a 96 well plate purified using this method.

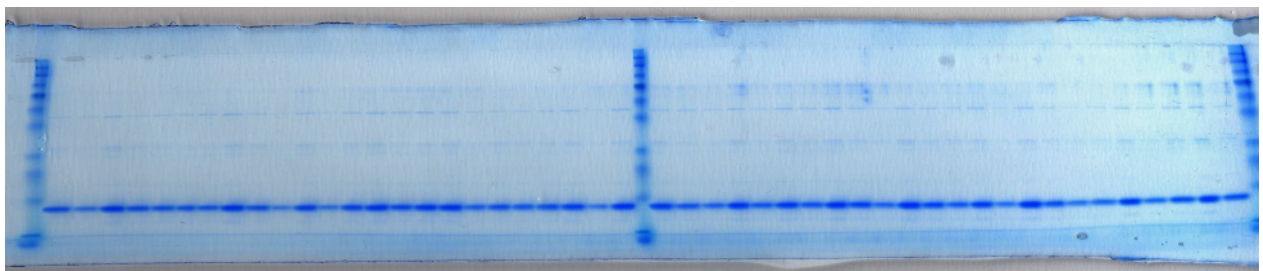


Figure 3: SDS-PAGE of 48 randomly chosen tagged proteins, purified on TheOnyx

Conclusions

- Magnetic bead separation provides a convenient and cost-effective means of purifying recombinant proteins.
- On a robotic system the process bottleneck following cell growth and protein production is alleviated.
- Isolated proteins maintain their fully functional conformation due to the small size of the 6xHis tags, and therefore are ideal for the downstream analysis of protein-protein or protein-DNA interactions.
- The time taken to process 96 samples was reduced by 30 minutes on TheOnyx compared with the previous robotic protocol. Lysis and binding of the protein remain the two most time consuming steps.
- The protocol performed on TheOnyx resulted in improved purity of the isolated proteins.

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