Automated purification of 5 mL lysates using dispersive solid phase extraction in pipettes

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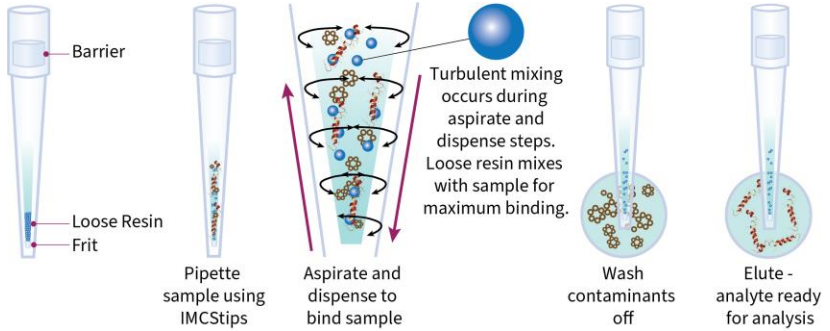
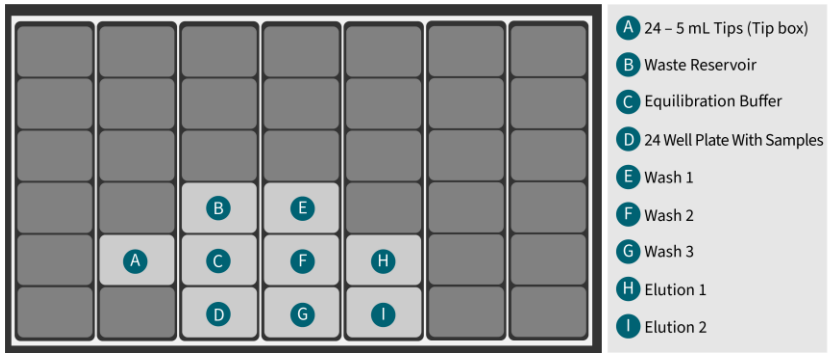
Introduction



- Affinity purification using 0.5 mL resin bed in 5 mL pipette tip
- Dispersive solid-phase extraction (dSPE) is loose resin in tip
- Up to 35 mg of recombinant protein (GFP) purified
- Processing of 5 mL lysate in 24-well sample plates in a batch
- Programmed on a single liquid handler to simplify workflows
- Program takes < 1 hour to purify 24 x 5 mL samples

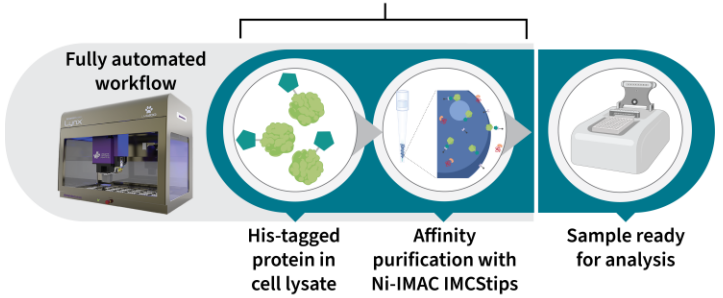
Instrumentation

- 5 mL IMCStips containing 500 µL Ni-IMAC (Cytiva) resin was used to capture and recover his-tagged green fluorescent protein (GFP) from 5 mL samples.
- Protein was quantified based on A280 using NanoDrop
- Protein purity was visualized by SDS-PAGE
- Dynamic Devices Lynx 1200 deck layout is shown below.

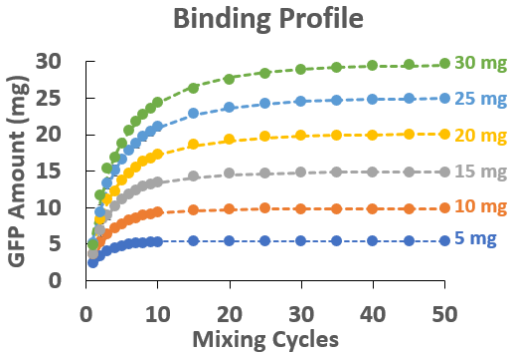


Methodology

Total processing time: < 1 hour

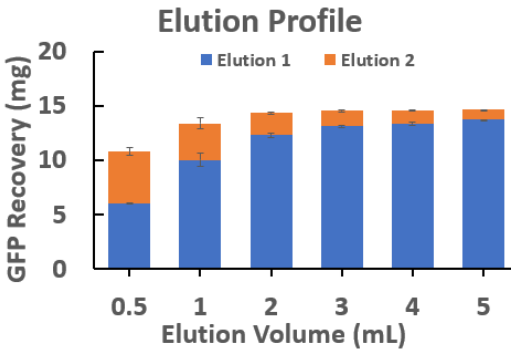


Result



Capture of GFP is fast in the first 10 binding cycles due to GFP's small molecular weight (27 kDa) and fast mass transfer

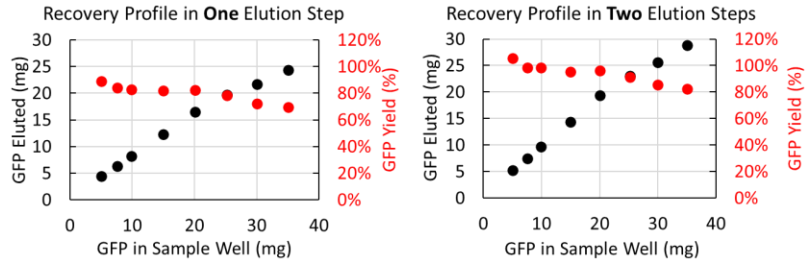
99% of protein is bound when there is ≤ 20 mg per well by 20th mixing cycles. At greater protein amounts, the number mixing cycles also increases to achieve similar binding efficiency.



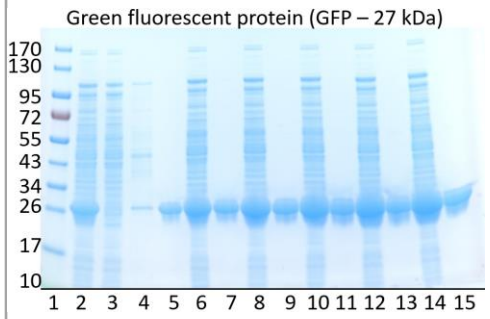
Elution volumes were tested on tips bound with 15 mg of GFP. Recovery percent plateaus at 2 mL and most of the bound fraction is recovered with 2 elutions.

Protein Recovery Profile

Recovery of GFP from bacterial cell lysate at different GFP load amounts, using one or two elutions. Each elution used 2 mL.



Purity of recovered GFP from different GFP load amounts



1	Ladder	9	20 mg - E1
2	10 mg - Sample	10	25 mg – S
3	10 mg - FT	11	25 mg - E1
4	10 mg - Wash 2	12	30 mg – S
5	10 mg – E1	13	30 mg- E1
6	15 mg – Sample	14	35 mg – S
7	15 mg - E1	15	35 mg - E1
8	20 mg - Sample		

S: Sample; FT: Flow through; E1: Elution 1

Conclusion

- The automated program recovered > 90% for samples up to 25 mg of GFP in 5 mL lysate.
- Recovery percentage slightly decreases with ≥ 30 mg GFP.
- Highly reproducible with < 3% recovery error.
- Based on SDS PAGE, GFP was > 90% pure.
- Future direction using multi-head or reagent dispenser for higher throughput will be explored.

Reference

1. P.A. Kates, J.J. Tomashek, D. A. Miles, L. A. Lee, *BioTechniques* ***2020***, 68, 148-154