



Chromatography Resin & Buffer Screening

A High-Throughput Platform for Parallel Evaluation of Chromatography Resins and Buffers in Recombinant Protein Purification

Dr. Neta Agmon, Meital Pinchas, Vivian Shemesh, Oz Reuveni, Hadar Fairstein | Alagene Ltd
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Email: info@alagene.co.il, Website: www.alagene.co.il

Executive Summary

Downstream process development remains one of the slowest and most resource-intensive stages in bringing a recombinant protein to the clinic or to market. Selecting the right chromatography resin and the right binding, wash, and elution buffers is a combinatorial problem: even a modest campaign can involve dozens of resin chemistries multiplied by dozens of buffer conditions. Screened one packed column at a time, this work consumes weeks of scientist time and tens of milligrams of precious material before a single lead condition is confirmed.

We have developed a **high-throughput (HTP) purification screening platform** that collapses this bottleneck. Using resin slurries dispensed into standard 96-well plates and driven entirely by an automated liquid handler, the platform evaluates **tens of resin-and-buffer combinations in parallel within a single working day**, using a fraction of the material a conventional column screen requires. The result is a data-rich yield-and-purity landscape that turns an intractable search space into a ranked short list of development-ready conditions.

This document describes the platform, its workflow, the analytics it produces, and the value it delivers to process-development teams and their partners.

1. The Problem: A Combinatorial Search With an Expensive Unit of Work

Purifying a recombinant protein to the required purity and yield is rarely a matter of picking a single "best" resin. Performance depends on the interaction between:

- **Resin chemistry** — affinity (e.g. Protein A), ion exchange, multimodal, hydrophobic interaction, and immobilized-metal affinity (IMAC) media, each available in multiple commercial grades;
- **Buffer conditions** — pH, ionic strength, and additives (arginine, salt, chaotropes) that govern binding capacity, selectivity, and elution behavior;
- **The specific molecule** — every construct behaves differently, so screening cannot be fully inherited from prior programs.

The mathematics are unforgiving. Ten resins against eight buffer conditions is already 80 experiments. Run as individual packed columns, each requiring equilibration, load, wash, elution, regeneration, and analytics, a screen of this size typically spans **six to eight weeks** and consumes **tens of milligrams** of purified or semi-purified feedstock — material that, early in development, may simply not exist in that quantity.

The consequence is that teams under time pressure screen too few conditions, lock in a resin early, and discover capacity, aggregation, or selectivity limitations later — when changing course is far more expensive.

2. The Platform: 96 Conditions on One Deck

Our platform reframes resin and buffer screening as a **parallel, miniaturized, automation-native** experiment rather than a serial one.

At its core is a resin-slurry batch-binding format in a **96-well plate**. Each well is an independent micro-purification: a defined volume of resin slurry receives a lysate or media and a specific buffer condition, and the bind–wash–elute cycle is performed in place using pipetting, liquid transfer, and centrifugation, with the product recovered by pipetting the elution fraction. Because every well is addressed independently, a single plate can host the **entire resin × buffer matrix** at once.

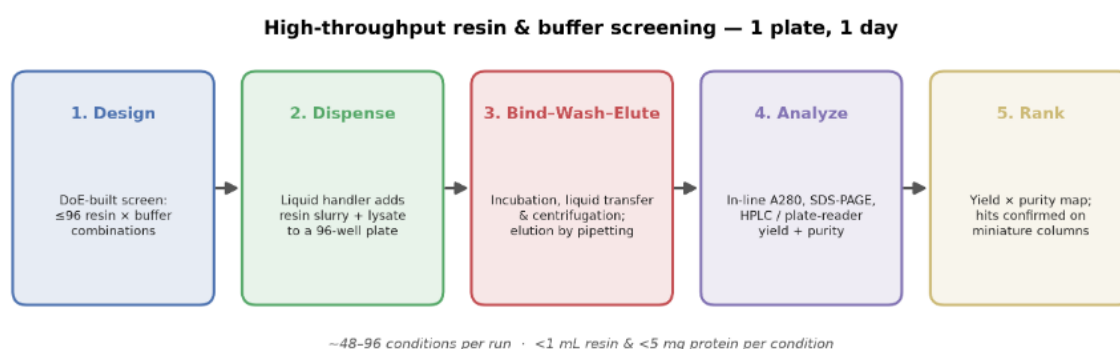


Figure 1. End-to-end workflow. A screen is designed as a resin × buffer matrix (optionally structured by design-of-experiments), dispensed by the liquid handler into a standard 96-well plate, processed through bind–wash–elute cycles using pipetting, liquid transfer, and centrifugation, and read out for yield and purity. Hits are ranked and forwarded to miniature-column confirmation. A full matrix runs in a single day using less than 1 mL of resin and under 5 mg of protein per condition.

Key characteristics:

- **Throughput:** 48–96 independent conditions per run.
- **Material economy:** sub-milliliter resin beds and single-digit-milligram protein loads per condition — an order of magnitude less than column screening.
- **Automation:** liquid handling, incubation, centrifugation, and plate-reader analytics run with minimal hands-on time and high step-to-step reproducibility.
- **Flexibility:** any resin available as a slurry can be screened side by side, allowing affinity, ion-exchange, multimodal, and IMAC chemistries to be compared in the same experiment.

3. Rich, Decision-Ready Analytics

Miniaturization is only useful if the readout is trustworthy. The platform pairs each well with quantitative analytics:

- **Recovery yield** from in-line A280 and plate-reader quantitation of load, flow-through, wash, and elution fractions;
- **Product purity** from SDS-PAGE, analytical HPLC/UPLC, and where relevant SEC for aggregate content;
- **Mass balance** across all fractions to flag non-specific binding or on-column losses.

The primary output is a **performance landscape** across the full matrix — the single view that makes the platform's parallelism actionable.

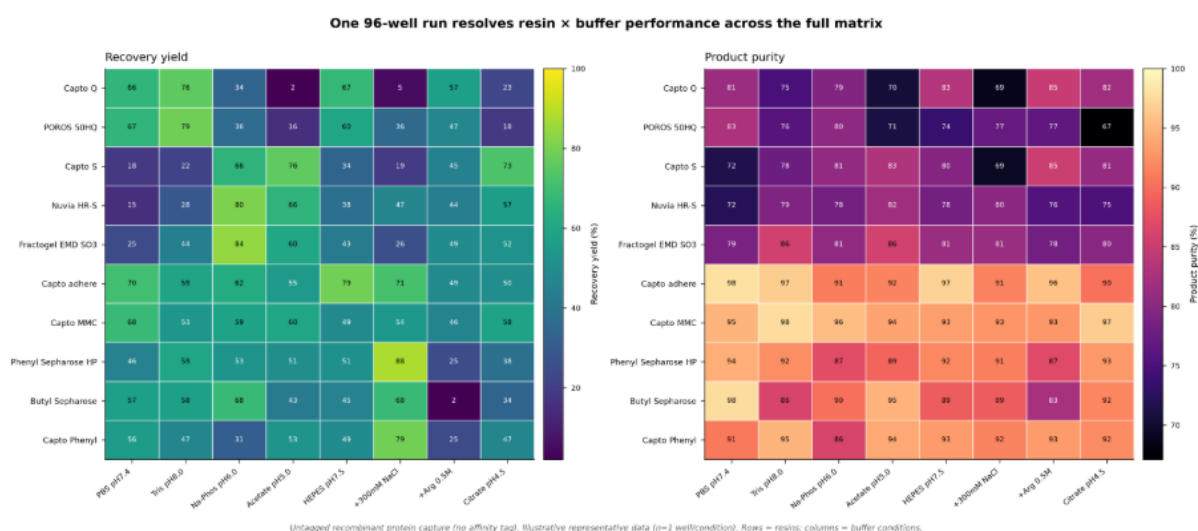


Figure 2. One run resolves the whole matrix. Recovery yield (left) and product purity (right) for ten resins against eight buffer conditions, screening the capture step for an ****untagged recombinant protein**** — one with no affinity tag, so capture must exploit the molecule's own surface properties — in a single 96-well run. The heatmaps immediately reveal chemistry-level structure: hydrophobic-interaction (HIC) media binding sharply under high-salt conditions, cation exchangers favoring low-pH/low-salt conditions, anion exchangers the opposite, and multimodal media binding broadly across the matrix. They pinpoint the specific wells that combine high recovery with high purity. Illustrative representative data; one well per condition.

Because yield and purity trade off against one another, the decisive analysis is a **joint selection map** that applies development thresholds to the whole dataset at once.

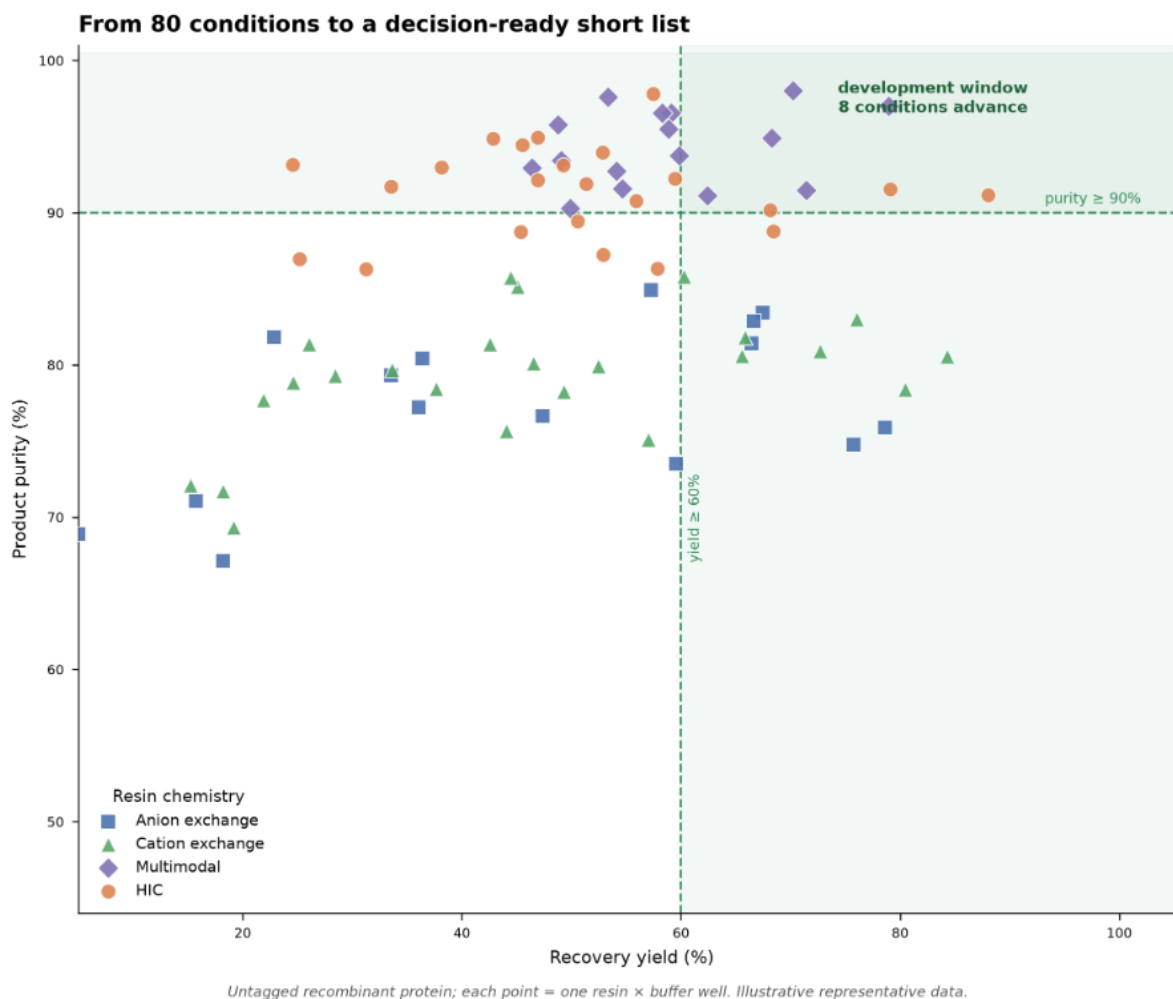


Figure 3. From 80 conditions to a short list. Every resin × buffer well for the untagged-protein capture, plotted by recovery yield and product purity and colored by resin chemistry (anion exchange, cation exchange, multimodal, HIC). A development window (here, yield ≥ 60% and purity ≥ 90%) isolates the conditions worth advancing — here concentrated in the ****multimodal and HIC**** media, the chemistries that deliver single-step selectivity when no affinity handle is available. What would otherwise be 80 separate column experiments becomes a single, defensible ranking in one view. Illustrative representative data.

4. The Value: Weeks Become a Day, Milligrams Become Micrograms

The platform's benefit is not incremental — it is a step change in the economics of downstream development.

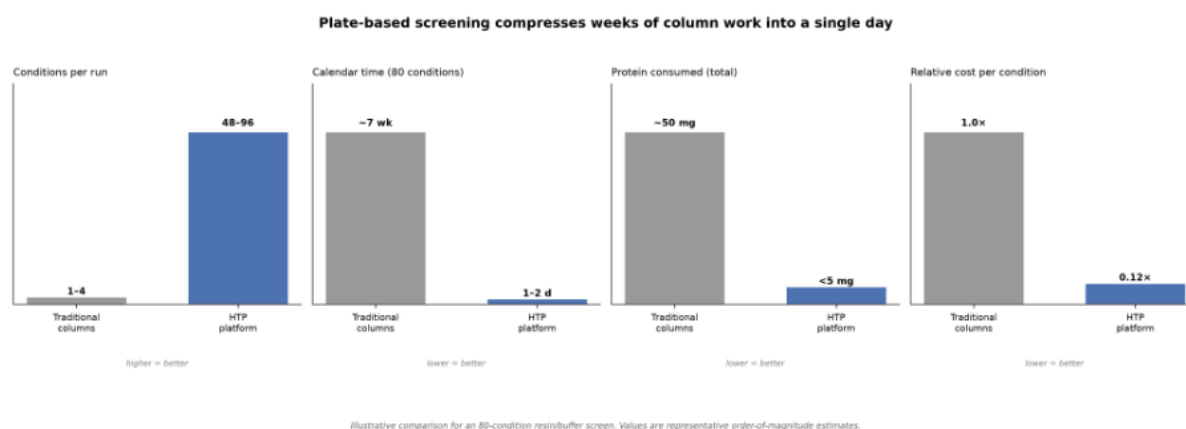


Figure 4. Order-of-magnitude gains on every axis that matters. For an 80-condition resin/buffer screen, the plate-based platform increases conditions per run, compresses calendar time from weeks to a day or two, cuts total protein consumption by roughly ten-fold, and reduces cost per condition by nearly an order of magnitude. Illustrative representative comparison.

Translated into program terms:

- **Speed:** resin/buffer selection moves off the critical path. A screen that gated a program for six to eight weeks now returns a ranked answer in one to two days.
- **Material:** screening becomes feasible at the earliest stages, when feedstock is scarce — reducing the temptation to lock in a resin on the basis of too little data.
- **Coverage:** because the marginal cost of an extra well is low, teams screen the *full* space rather than a hopeful subset, lowering the risk of late-stage surprises.
- **Cost:** less resin, less protein, and less scientist time per condition compound into a substantially cheaper development campaign.

5. Fitting Into a Development Funnel

The platform is designed as the **broad top of a screening funnel**, not a replacement for confirmatory chromatography:

1. **Screen (this platform):** cast a wide net across resins and buffers in 96-well plates; rank by yield and purity.
2. **Confirm:** advance the top handful of conditions to miniature packed columns (e.g. pre-packed micro-columns) to verify behavior under flow — breakthrough capacity, gradient elution, and resolution.

3. **Scale:** carry the confirmed condition into bench-scale column development with high confidence and a documented rationale.

This tiered approach preserves the predictive value of true column chromatography while removing the combinatorial burden from its most expensive stage.

6. Applications

- **Early process development** for monoclonal antibodies, fusion proteins, enzymes, and non-antibody scaffolds.
- **Capture-step selection**, including Protein A alternatives and platform-fit assessment for new modalities.
- **Polishing-step optimization** across ion-exchange and multimodal chemistries.
- **Buffer and additive screening** for challenging molecules prone to aggregation or non-specific binding.
- **Resin lifetime and re-use studies**, and side-by-side benchmarking of new commercial media.
- **Technology transfer and troubleshooting**, where a rapid re-screen can resolve a stalled process.

7. Pilot Study: Five Resins Across Eight pH Conditions

To demonstrate the platform in practice, we ran a pilot screen of **five chromatography resins against eight pH conditions** (pH 3, 4, 5, 6, 7, 7.5, 8, and 9) — a 40-condition matrix executed in a single 96-well run. Eluate protein was quantified by Bradford assay, so higher signal indicates greater recovery in the elution fraction.



Pilot screen: 5 resins × 8 pH conditions, one Bradford reading per well.

Figure 5. A single run localizes the optimum. Bradford signal for the eluate of each resin × pH combination. A sharp recovery hotspot emerges at ****pH 5 for Resins 3, 4, and 5**** (signals of 0.95, 1.00, and 0.99 respectively) — roughly 2–2.5× the background seen across most other conditions. The pattern is unambiguous and would have required 40 separate column runs to establish conventionally.

The per-resin pH profiles make the underlying behavior clear and separate the panel into two distinct groups.

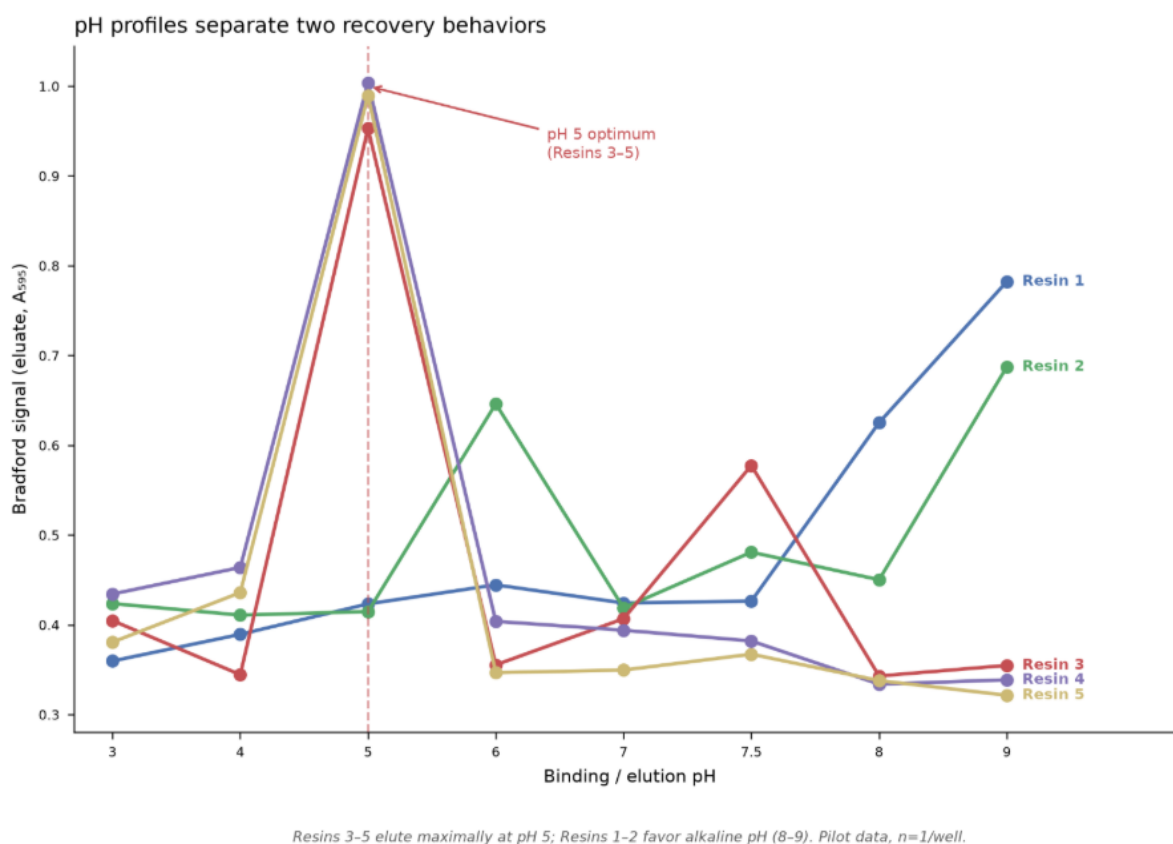


Figure 6. Two recovery behaviors, one experiment. Resins 3, 4, and 5 share a pronounced, narrow optimum at pH 5 and low recovery elsewhere — the signature of a pH-sensitive elution well suited to a defined process step. Resins 1 and 2 behave oppositely, giving their highest recovery under alkaline conditions (pH 8–9). A single day's screen thus not only names the lead condition (Resin 4 at pH 5) but also reveals the mechanistic split among the resins tested — information that directly informs which chemistry to advance and over what pH range to develop it.

What the pilot shows: the platform converts a 40-experiment design into one plate, resolves a clear winner (**Resin 4 at pH 5**) alongside two credible backups (Resins 5 and 3 at the same pH), and simultaneously characterizes the full pH-response landscape of every resin — the kind of decision-ready result that would otherwise take weeks of serial column work to assemble.

Proof-of-concept confirmation. The three lead conditions were carried forward and analyzed by SDS-PAGE, resolving the **load**, **unbound (flow-through)**, and **eluate** fractions for each resin at its optimal pH.

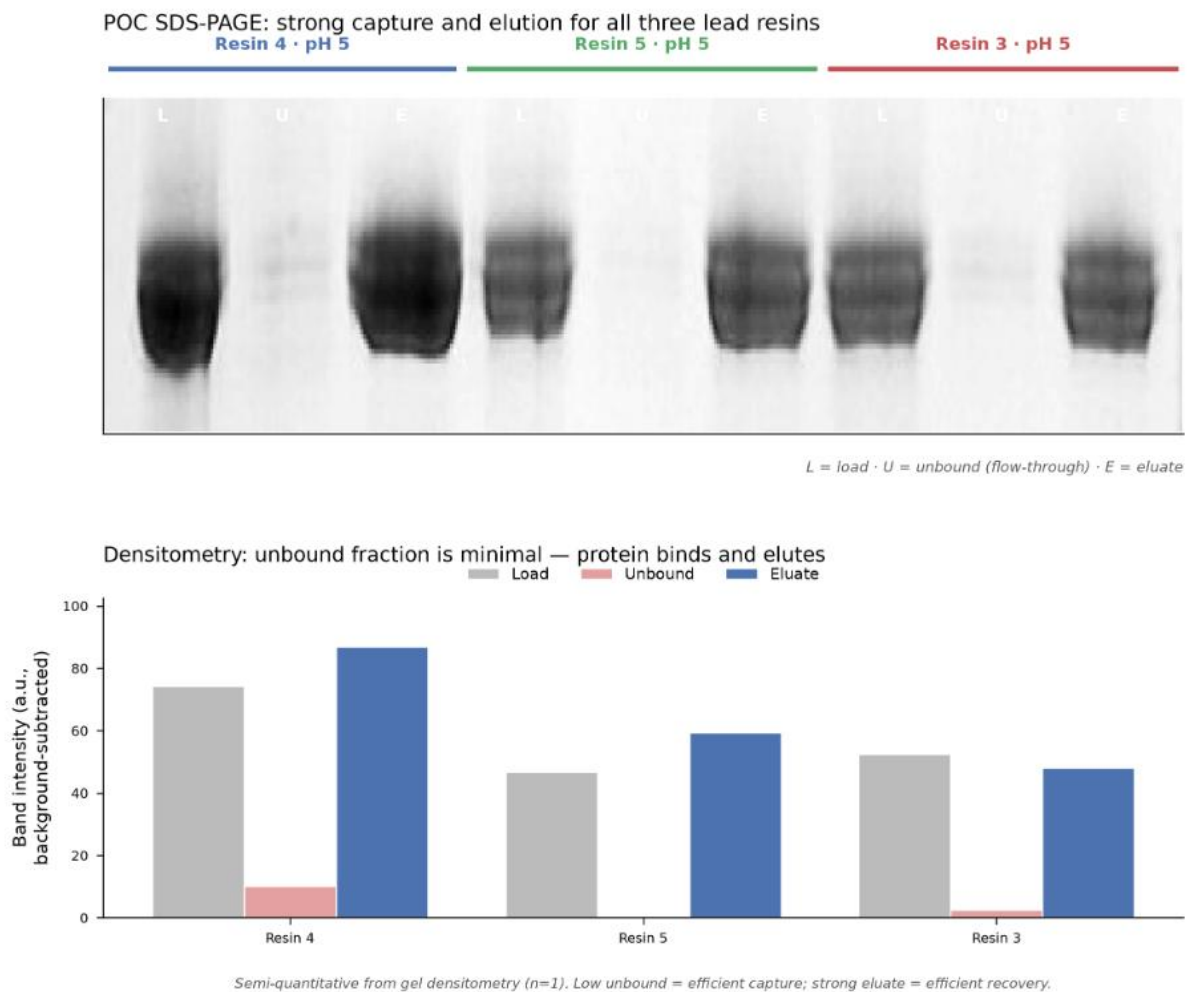


Figure 7. The gel confirms the mechanism behind the screen. For all three lead resins (Resin 4, 5, and 3 at pH 5), the load lane carries a strong target band, the unbound lane is nearly empty, and the eluate lane recovers a strong band. The near-absence of protein in the flow-through demonstrates ****efficient capture**** — the resin binds essentially all of the loaded target — while the strong eluate band demonstrates ****efficient recovery****. Densitometry (lower panel) puts the unbound fraction at roughly 0–15% of the load across the three resins. This is the textbook signature of a well-behaved capture step and validates the Bradford-based screen: the conditions the plate flagged as best do, in fact, bind and release the protein cleanly. Semi-quantitative densitometry, n=1.

8. Conclusion

Resin and buffer selection has long been treated as a serial, column-by-column search — thorough but slow, and expensive in exactly the material that is scarcest early in a program. By moving the screen into standard 96-well plates on an automated liquid handler, our platform makes the full resin × buffer matrix a single day's experiment, delivering a decision-ready yield-and-purity landscape at a fraction of the time, material, and cost. The outcome is faster, better-informed downstream development — and a shorter path from clone to purified product.