# The Forgotten Immunoglobulin

## LigaTrap™ Technologies and Advancements in IgM Purification



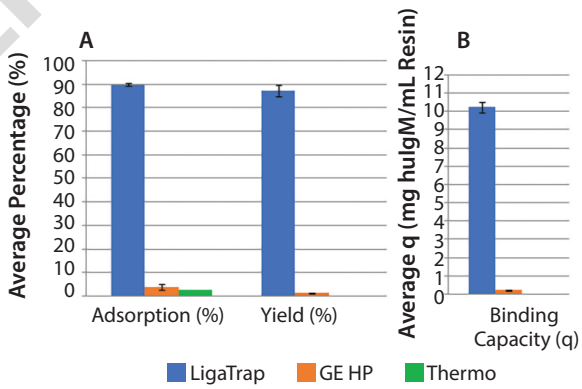
It's been over 30 years since the identification and commercialization of protein A. Since its inception, protein A has served as the pinnacle purification platform for the immunoglobulin G (IgG) subclass of antibodies, both monoclonal and polyclonal forms. The introduction of protein A paved the way for researchers and large pharmaceutical manufacturers to develop and commercialize IgG-based therapeutics at both small and large scales, provided by its relative ease of use and well-characterized chromatographic attributes. Unfortunately, the gold standard affinity ligand, protein A, does not bind to all immunoglobulin subclasses, including immunoglobulin M (IgM), thereby forcing those working with IgM to find alternative purification strategies and leaving IgM to hitchhike on that "yellow brick road" that protein A offers so readily for IgG.

Moreover, little to no advancements have been made in developing a robust IgM purification platform to date. Therefore, the emergence of IgM-based therapeutics has been hindered. For this reason, it is not surprising that IgM might sometimes be considered "the forgotten immunoglobulin", relative to its famous immunoglobulin counterpart, IgG. LigaTrap™ Technologies is changing this perception of IgM by bringing to market the first affinity ligands for IgM, offering a new platform for IgM purification, one that can be the missing piece to elevate IgM research and drug discovery to a whole new level.

The immunoglobulin market predominantly consists of monoclonal antibodies (MAbs) and is expected to be US$125 billion by 2020 with the development and advancement of MAb-based therapeutics to treat specific diseases. Multiple reports have revealed a strong interest in IgM as a potential therapeutic candidate for its ability to demonstrate promising anticancer activity, restore immune homeostasis, and play an important role in immune-surveillance mechanisms against tumors (**1–6**). For those reasons, there is a rapidly growing field of research using IgM in the areas of cancer, diabetes, immune response, pneumonia, and sepsis. Therefore, several industrial endeavors have been made to identify a standard platform for IgM purification, including ammonium sulfate precipitation, gel filtration, anion exchange, and affinity chromatography. However, those traditional IgM purification processes can be time consuming (and expensive) and often require multiple processing steps to achieve adequate purity and concentration.

LigaTrap™ TECHNOLOGIES

**Figure 1:** Statistical analysis of all three human IgM purification resins in terms of % adsorption, % yield, and binding capacity; averages were based on calculations from each resin being tested in triplicate. (A) Bars represent the average percentages (±95% confidence interval) of human IgM adsorption and yield for each purification resin type. (B) Bars represent the average static binding capacity for human IgM (±95% confidence interval) for each purification resin type.




To address such limitations of modest advancements in IgM purification and to meet future market demands, LigaTrap™ Technologies has developed a novel and very efficient alternative solution. It consists of chromatography resins using peptoid-based ligands to capture IgMs, referred to as LigaTrap™ human IgM purification resin. Peptoids are peptide mimetic compounds that have been engineered to bind with high affinity to IgM, allowing for high binding capacity, high selectivity, and reasonable cost. They provide for lower wholesale and retail prices than those related to the use of currently available IgM purification systems.

### The Study

LigaTrap™ Human IgM purification resin and two commercial IgM purification resins (Table 1) were tested in triplicate for their

**Equations 1–4:**

**Equation 1**

$$\text{Adsorption (\%)} = 100 \times \frac{Mass_{bound}}{Mass_{load}}$$

**Equation 2**

$$\text{Static binding capacity (q)} = \frac{V_{sample} \times (c_o - c_{eq})}{V_{resin}}$$

**Equation 3**

$$\text{Yield (\%)} = 100 \times \frac{c_{elute} \times V_{relute}}{c_{load} \times V_{load}}$$

**Equation 4**

$$\text{Mass balance (\%)} = 100 \times \frac{Mass_{bound} + Mass_{unbound}}{Mass_{load}}$$

**Table 1:** Chromatography resins tested

| Commercial IgM Purification Resin | Cat. Number | Abbreviation |
|---|---|---|
| LigaTrap™ human IgM purification resin | LT-143 | Ligatrap |
| GE Healthcare HiTrap™ IgM purification HP | 17-5110-01 | GE HP |
| Pierce™ IgM purification kit | 44897 | Thermo |

**Table 2:** Composition of proteins in sample load (starting material)

| Component | Concentration |
|---|---|
| 5% fetal bovine serum | 2.90 mg/mL |
| Human IgM | 2.25 mg/mL |
| Bovine, albumin | 1.85 mg/mL |
| Chicken, albumin | 1.85 mg/mL |
| B-Galactosidase | 25 µg/mL |
| Glycerladehyde-3-phosphate-dehydrogenase | 250 µg/mL |
| Phosphorylase-b | 40 µg/mL |
| Carbonic anhydrase | 375 µg/mL |

ability to purify human IgM from a sample matrix containing 5% fetal bovine serum (FBS) and several other competing proteins (Table 2).

Purification was done in spin-column format with 75 mg of resin in each spin column. Sample matrix as described in Table 2 was applied to all resins such that a final loading of 12 mg of IgM per mL of resin was achieved. All resins were tested using buffers and chromatographic procedures recommended by their respective manufacturers. The flow-through and elution fractions for all resins were evaluated to determine the performance of each respective resin.

The concentration of human IgM in all fractions was measured by sandwich enzyme-linked immunosorbent assay (ELISA). Purification quality performance was measured in terms of adsorption (%), binding capacity (q), yield (%), and purity (%), and their respective values were established as follows. As shown in Equation 1, adsorption (%) = percentage of total mass of IgM bound to the solid phase) and was calculated from the amount of IgM bound and the amount loaded. In Equation 2, static binding capacities of IgM were determined for each

**Figure 2:** Coomassie-stained SDS-PAGE in reducing conditions; equivalent volumes of the flowthrough and elute fractions were analyzed. 1.7 µg of human IgM standard was loaded in the gel. The sample load was run at equivalent volume to the flowthrough fractions. Arrows indicate the position of heavy-chain (68 kDa) and light-chain (26 kDa) human IgM.

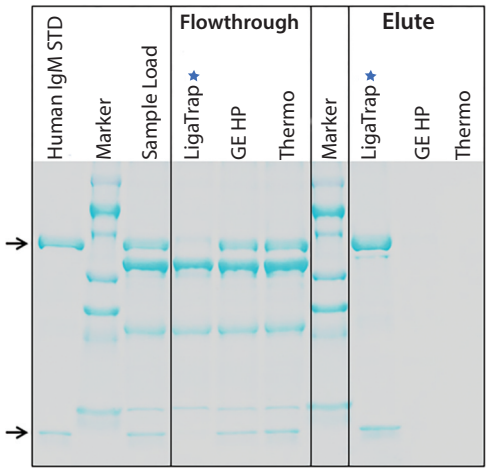
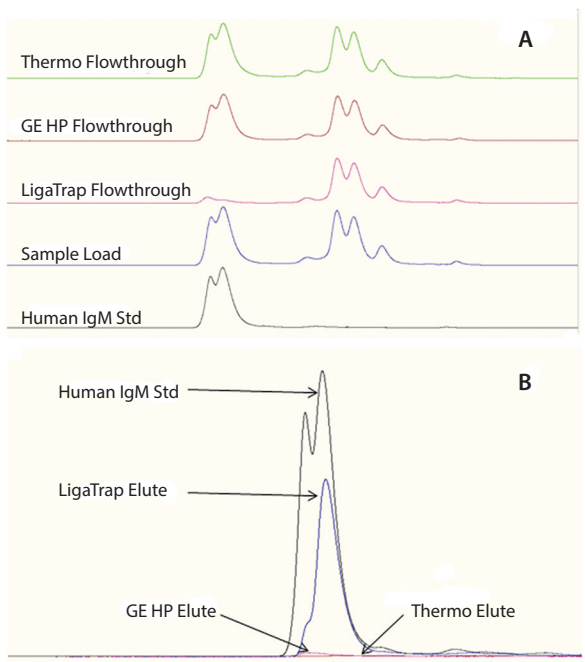



**Figure 3:** Analytical SEC-HPLC profiles of flowthrough and elute fractions; column: MabPac SEC-1 (30 cm); mobile phase: PBS; flow rate: 0.15 mL/min; wavelength: 280 nm; injection: 10 µL. (A) Flowthrough fraction analysis, including the sample load (starting material) and the human IgM reference standard (2.2 µg); (B) elution fraction analysis, including the human IgM reference standard (2.2 µg).




purification methodology. Then yield (%) was calculated from the amount of IgM eluted from the solid phase (resin) and the amount of IgM loaded, as shown in Equation 3. Purity (%) was assessed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and size-exclusion chromatography–high-performance chromatography (SEC-HPLC) analysis. Equation 4 shows how mass balance (%) =

(percentage of total mass recovered) can be calculated from the amount of IgM bound, unbound, and the amount loaded.

## Results and Discussion

As seen in Figure 1, the average static binding capacity (q ± CI95%) of human IgM was 10.3 ± 0.3 mg/mL resin for LigaTrap™ resins. The data showed that the competitors' resins were unable to bind efficiently to IgM, having an average binding capacity estimated <0.2 mg/mL resin, despite GE's reported claim of 5 mg/mL resin. In an attempt to increase IgM binding for GE HP, we conducted several additional studies, including various binding conditions recommended by GE, and were unsuccessful in demonstrating any reasonable IgM binding using the GE resin (data not shown). This study clearly demonstrates that LigaTrap™ IgM purification resin provides an optimal matrix for IgM adsorption (90.0 ± 0.8%) and therefore allows for high binding capacity and efficient sample recovery, significantly higher than those of competitor resins.

The percentages of yield as shown in Figure 1 were found to be 87%, 1.5%, and 0.2% for LigaTrap™, GE HP, and Thermo resins, respectively. This is based on a 12 mg IgM per mL resin loading of the resins. The average mass balance (± CI95%) was 98 ± 2% for all the purification methods used in this study. Such high mass balances validate the integrity of IgM quantification in these experiments and thus validate all IgM concentration-dependent calculations for this study, including yield (%) and static binding capacity (q).

The purity of the eluted IgM was assessed by SDS-PAGE stained with Coomassie blue dye and HPLC using SEC. The SDS-PAGE analysis was conducted under reducing conditions. Therefore, both heavy (~68 kDa) and light chains (~26 kDa) for IgM are observed where IgM is present. In Figure 2, SDS-PAGE analysis revealed for LigaTrap™ resins a significant adsorption of IgM in the flow-through fractions, evidenced by a reduced band intensity where IgM heavy and light chains should be in the gel, whereas both GE HP and Thermo resins showed very little adsorption of IgM. Furthermore, highly purified IgM was observed in the elution fraction (>97% pure, based on densitometry) for the LigaTrap™ resin. IgM in the elution fraction was faintly detected for GE HP resins and below detection limits for Thermo resins.

HPLC analysis (Figure 3) revealed similar findings to the SDS-PAGE analysis in that the LigaTrap™ resins adsorbed the majority of the IgM in the sample and eluted IgM at a very high purity (97%). Again, GE HP and Thermo resins failed to bind any significant amount of IgM and therefore had undetectable levels of IgM in the elute fractions.

## Conclusions

The LigaTrap™ IgM purification resin proved far superior to the GE HP and Thermo products tested in this study. LigaTrap™ resin purified more than 50- and 350-fold more IgM than GE and Thermo products, respectively. Additionally, the purity of the eluted IgM was extremely high, at 98%. Finally, the binding capacity was determined to be 10.3 mg IgM per mL resin for the LigaTrap™ resin. Our recent findings showed high binding affinity of LigaTrap™ resins to purify both lambda (γ) and kappa (κ) IgM isoforms (data not shown). The "forgotten immunoglobulin", IgM, is no longer. LigaTrap™ Technologies has now given IgM researchers a new-found hope by developing this novel IgM purification platform, which we hope will lead to a revolutionary growth in the discovery of life changing IgM-based therapeutics in the near future.

## But Wait, There's More . . .

In addition to the LigaTrap™ IgM purification resin presented in this study, LigaTrap offers affinity resins, as well as other immunoglobulin purification platforms such as human IgG (all isotypes), human IgA, mouse IgG (all isotypes), rat IgG, sheep IgG, goat IgG, sheep IgG, llama IgG (all isotypes), and chicken IgY. After a few simple steps (loading, washing, and elution), the antibodies are recovered in a highly pure form. Moreover, unlike traditional protein A and protein G purifications, antibody is eluted with a milder pH (pH 4.0), for a gentler elution that is more favorable for antibodies that are prone to aggregation under low pH conditions (an attribute particularly desirable when purifying monoclonal antibodies). More information about the other antibody purification platforms, IgM purification, or batch and column attributes is available at www.ligatrap.com or by requesting LigaTrap technical resources at info@ligatrap.com.

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