



# Protein Purification



Chondrex, Inc offers a range of resins for protein purification. The following provides a summary on different protein purification mechanisms in order to help you choose the appropriate resins for your research and study purposes.

## 1. Ion-exchange Chromatography (1, 2)

Each protein has a net charge, called the isoelectric point (pI), determined by its primary amino acid sequences. This charge property allows proteins to selectively bind to resins with opposite charges in ion-exchange chromatography. This technique utilizes the following principle - positively charged resins (anion exchange: DEAE and Q), or negatively charged resins (cation exchange: CM ). Then, alterations in pH or salt gradients facilitate the elution of proteins from the resin.

## 2. Affinity Chromatography (3)

Affinity chromatography is a highly effective method for the purification of specific proteins through biological interactions, leveraging the strong, yet reversible, binding between molecules. This method immobilizes one of the interacting proteins on a chromatographic resin, allowing for the selective binding and subsequent elution of the target protein by altering conditions, such as pH or salt concentration.

## Protein Purification Resins

|                                 | Ion Exchange Purification   | Antibody Affinity Purification   | His-tag Protein Affinity Purification  |
|---------------------------------|---|--|--|
| Ligand or Chemistry (Catalog #) | DEAE: 9079 (5 ml)<br>Q: 9081 (5 ml)<br>CM: 9080 (5 ml)  | Protein A: 9076 (1 ml)<br>Protein G: 9077 (1 ml)   | Ni-IMAC: 9078 (5 ml)   |
| Base Support                    | DEAE: Highly cross-linked 6% Sepharose<br>Q: Highly cross-linked 6% Sepharose<br>CM: Highly cross-linked 6% Sepharose   | Protein A: Rigid, highly cross-linked Agarose<br>Protein G: Highly cross-linked 4% Sepharose | Highly cross-linked 6% Sepharose   |
| Formats                         | A drip column / column chromatography   | A drip column / column chromatography  | A drip column / column chromatography  |
| Binding Capacity (mg/ml)        | DEAE: Weak anion exchange<br>110-160 $\mu\text{mol Cl}^-$ /ml media<br><br>Q: Strong anion exchange<br>180-250 $\mu\text{mol Cl}^-$ /ml media<br><br>CM: Weak cation exchange<br>90-130 $\mu\text{mol H}^+$ /ml media | Protein A: 60 mg (human IgG)/ml media<br><br>Protein G: $\geq 20$ mg (human IgG)/ml media    | $\geq 40$ mg (His-tag protein)/ml media<br><br>16-23 $\mu\text{mol (Ni}^{2+})$ /ml media |
| Equivalent Products             | DEAE Sepharose 4FF<br>Q Sepharose 4FF<br>CM Sepharose 4FF   | MabSelect sure LX<br>Protein G Sepharose 4FF   | Ni Sepharose 4FF   |



# Protein A & Protein G



## Affinity of Protein A/G for Immunoglobulin Types from Different Species

Protein A and Protein G exhibit specific binding affinities for various immunoglobulin (Ig) subtypes, subclasses, and species, which are determined by the characteristics of the Ig Fc region. The details of their binding affinities are listed in the following chart that categorizes the interaction strengths of Protein A and G with immunoglobulin subclasses across different species.

| Species | Immunoglobulin | Binding to Protein A | Binding to Protein G |
|---------|----------------|----------------------|----------------------|
| Human   | IgG (normal)   | ++++                 | ++++                 |
|         | IgG1           | ++++                 | ++++                 |
|         | IgG2           | ++++                 | ++++                 |
|         | IgG3           | -                    | ++++                 |
|         | IgG4           | ++++                 | ++++                 |
|         | IgM            | -                    | -                    |
|         | IgA            | -                    | -                    |
|         | IgE            | -                    | -                    |
| Mouse   | IgG1           | +                    | ++++                 |
|         | IgG2a          | ++++                 | ++++                 |
|         | IgG2b          | +++                  | +++                  |
|         | IgG3           | ++                   | +++                  |
| Rat     | IgG1           | -                    | +                    |
|         | IgG2a          | -                    | ++++                 |
|         | IgG2b          | -                    | ++                   |
|         | IgG2c          | +                    | ++                   |
| Goat    | IgG            | +/-                  | ++                   |
| Rabbit  | IgG            | ++++                 | +++                  |
| Sheep   | IgG            | +/-                  | ++                   |

"-" : No binding, "+" : Weak binding, "++" : Binding, "+++": Moderate Binding, "++++" : Strong Binding

### References

1. P. M. Cummins, K. D. Rochfort, B. F. O'Connor, Ion-Exchange Chromatography: Basic Principles and Application. *Methods Mol. Biol.* **1485**, 209–223 (2017).
2. A. Jungbauer, R. Hahn, "Chapter 22 Ion-Exchange Chromatography" in *Methods in Enzymology*, R. R. Burgess, Deutscher, Murray P., Eds. (Academic Press, 2009) vol. 463, pp. 349–371.
3. M. Urh, D. Simpson, K. Zhao, Affinity chromatography: general methods. *Methods Enzymol.* **463**, 417–438 (2009).