

## Purification of Horse IgG<sub>T</sub> Using Macro-Prep® DEAE and CHT™ Ceramic Hydroxyapatite Type I Supports

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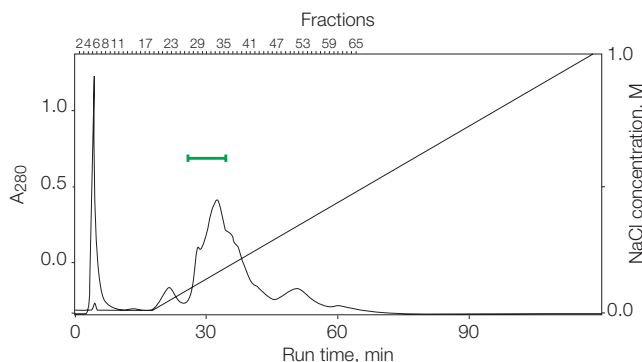
### Introduction

Horse immunoglobulin G consists of several subclasses distinguishable by their immunoelectrophoretic mobility, designated IgG<sub>a</sub>, IgG<sub>b</sub>, IgG<sub>c</sub>, IgG<sub>1</sub>, and IgG<sub>T</sub>. IgG<sub>T</sub> is the principal immunoglobulin class in which horse antitoxin antibody activity resides. Hyperimmunized horses produce large amounts of IgG<sub>T</sub>. IgG<sub>T</sub> is used therapeutically for treatment of life-threatening snake bites. Large amounts of antivenom serum are often needed to obtain a therapeutic effect, which increases the potential risk of serum-related allergic reactions (Fernandes et al. 1991). Apparently, complete purification of this immunoglobulin is essential to avoid allergic reactions in patients receiving this serum.

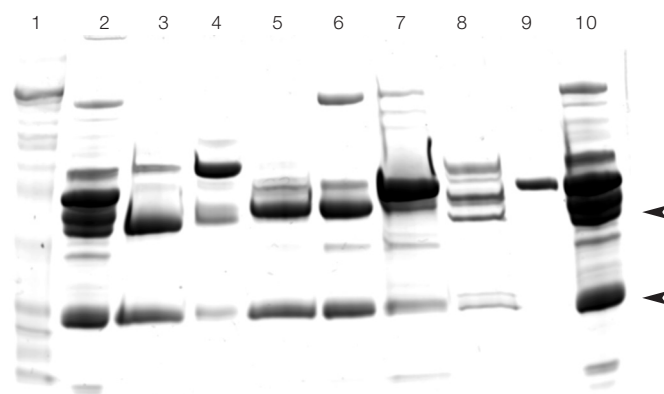
Previously, elaborate and time-consuming methods such as precipitation, dialysis, gel filtration, affinity chromatography, etc., were required to isolate and purify IgG<sub>T</sub> (Goudswaard et al. 1977). We describe here the development of a simple two-step procedure to purify IgG<sub>T</sub> from hyperimmunized horse plasma using Macro-Prep DEAE and CHT ceramic hydroxyapatite, Type I chromatographic supports.

### Methods and Results

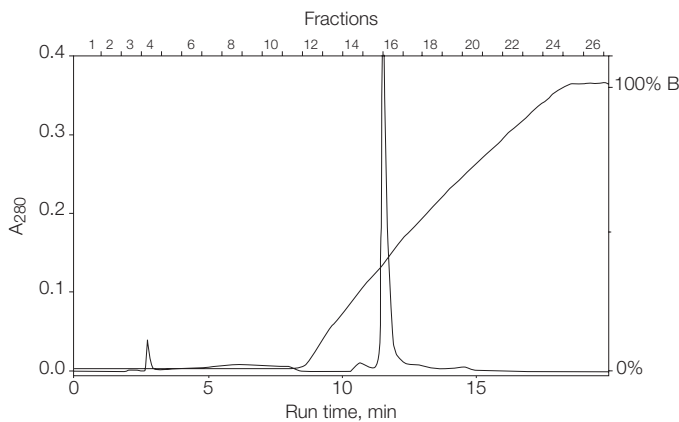
The horse plasma sample was filtered and loaded onto a Macro-Prep DEAE column, then eluted with a linear gradient of 0–1 M NaCl as described in Figure 1. Fractions were pooled and analyzed for protein purity by SDS-PAGE using 4–15% acrylamide gradient gels under reducing conditions. A Bethyl VET-RID (radial immunodiffusion) quantitation kit was used to specifically assay the pooled fractions for IgG<sub>T</sub> content. RID analysis was based on an antigen-antibody reaction, occurring in a support medium (agarose gel), that was visible as an opaque precipitin ring. Fractions 26–30 and 31–34 gave positive results; fractions 3–8, which constituted the unbound peak, contained most of the IgG (data not shown). SDS-PAGE analyses indicated fractions 26–30 and 31–34 were partially purified IgG<sub>T</sub> (Figure 2, lanes 5 and 6).



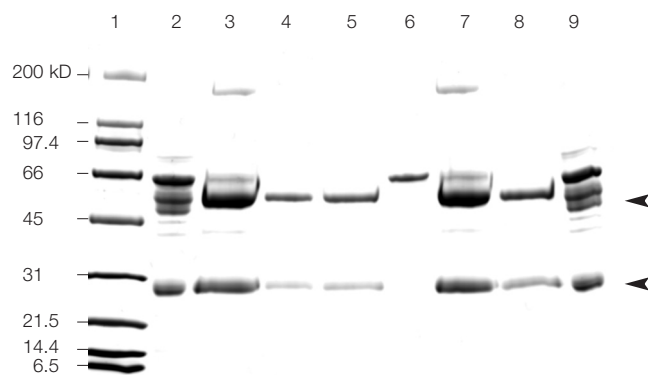
**Fig. 1. Purification of horse plasma on Macro-Prep DEAE column.** Horse plasma was loaded onto a 1.0 x 10 cm column equilibrated in binding buffer (10 mM sodium phosphate, pH 7.9) and washed with 2 column volumes of binding buffer. The column was then eluted with a linear gradient of 0–1 M NaCl in binding buffer for 20 column volumes. The flow rate was 2 ml/min and the fraction size was 2.0 ml; green bar indicates where IgG<sub>T</sub> eluted.



**Fig. 2. SDS-PAGE analysis of Macro-Prep DEAE fractions.** Lane 1, broad range MW standards; lane 2, horse plasma; lane 3, fractions 3–8; lane 4, fractions 20–25; lane 5, fractions 26–30; lane 6, fractions 31–34; lane 7, fractions 35–46; lane 8, fractions 47–56; lane 9, albumin standard; lane 10, horse plasma. Arrows indicate position of IgG.



**Fig. 3. Horse IgG<sub>T</sub> purification on Bio-Scale CHT2-I column.** The pooled IgG<sub>T</sub> fractions from the DEAE column (green bar in Figure 1) were loaded onto a 7 x 52 mm column in 5 mM sodium phosphate, pH 6.8 (buffer A), then eluted with a gradient of 0–100% buffer B (100 mM sodium phosphate + 1 M NaCl, pH 6.8) for 10 column volumes. The flow rate was 2 ml/min, and the fraction size was 1.5 ml.



**Fig. 4. SDS-PAGE summary analysis of the purification.** Lane 1, broad range MW standards; lanes 2 and 9, horse plasma; lanes 3 and 7, fractions 26–34 off DEAE column (Figure 1); lanes 4, 5, and 8, fraction 16 off CHT2-I column (Figure 3); lane 6, albumin standard. Arrows indicate position of IgG.

Fractions 26–34 (green bar in Figure 1) were pooled and loaded onto a Bio-Scale™ CHT2-I column (catalog #751-0021) previously equilibrated with 5 mM sodium phosphate buffer, pH 6.8. Horse IgG<sub>T</sub> was eluted with a linear gradient of 0–1 M NaCl, as described in Figure 3.

Fractions collected were analyzed for protein purity by SDS-PAGE using 4–15% gradient gels under reducing conditions. The major peak (fraction 16) showed 98–99% purity on the gel (Figure 4, lanes 4, 5, and 8).

### Discussion

A simple two-step process for the purification of horse IgG<sub>T</sub> directly from plasma was developed. This procedure should be readily scalable and avoids the use of ammonium sulfate or caprylic acid fractionation, which can denature some immunoglobulins.

During the course of these studies, it was discovered that enhancement of resolution on the CHT column was obtained by running a sodium chloride and a sodium phosphate gradient at the same time. Running either component alone resulted in broad peaks and only partial elution. Although the precise mechanism for this phenomenon is not clear, we can postulate that breaking both electrostatic and coordinate bonds simultaneously (at least for some macromolecules) provides significantly altered retention times and may also affect protein solubility and protein-protein interactions. This new method may have broader applications in macromolecule resolution.

### References

- Fernandes I et al., Isolation of IgG<sub>1</sub> from hyperimmune horse anti-snake venom serum: its protective ability, *Toxicon* 29, 1373–1379 (1991)
- Goudswaard J et al., Isolation of equine IgG(T) by hydrophobic interaction chromatography, *Immunochemistry* 14, 717–719 (1977)

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