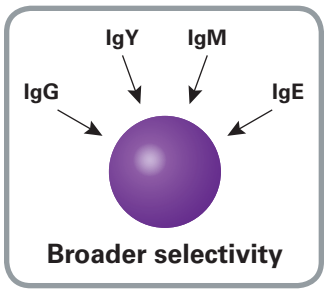
Thiophilic Antibody Purification Resins



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An efficient, versatile, and economical alternative to Protein A purification

Clontech's **Thiophilic-Uniflow** and **-Superflow Resins** provide highly stable purified antibodies with the following advantages over conventional Protein A antibody purification methods:

- Purification at neutral pH—avoid antibody aggregates
- High capacity (20–25 mg Ab/ml resin)—get high recoveries
- Broader selectivity—purify IgY, IgM, IgE, and scAb
- Fewer purification steps—save time
- Reusable resin—save money
- Higher stability of the purified product

Protein A vs. Thiophilic Resin

Historically, Protein A has been the preferred method of immunoglobulin purification. However, there are certain types of antibodies, such as the single-chain antibodies IgE, IgY, and IgM, that cannot be purified using Protein A. Thiophilic adsorption chromatography is ideal for these types of applications, as well as immunoglobulin purification in general.

In addition to providing broader selectivity and higher stability, thiophilic adsorption chromatography offers a faster protocol than Protein A under neutral buffer conditions (Figure 1). Purification with Thiophilic Resin allows elution in any buffer with pH 7.0. In contrast, Protein A requires elution in a buffer with pH lower than 7.0. Protein A also requires a dialysis step, which can be omitted with Thiophilic Resin.

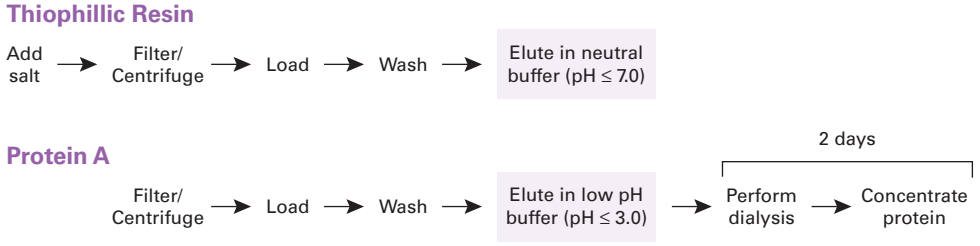

Figure 1. Thiophilic Resin purifies antibodies at neutral pH and in less time than Protein A.

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Thiophilic Ligand Allows Salt-Dependent Purification of a Wide Range of Antibody Types

Thiophilic adsorption chromatography (TAC) was developed by Porath *et al.* (1). TAC is a group-specific, salt-dependent purification technique with distinct adsorption affinity towards immunoglobulins and α2-macroglobulins. The term "thiophilic" refers to the affinity that proteins have for sulfone groups that lie in close proximity to thioether groups (Figure 2; 2). With this technique, protein adsorbs to a sulfone thioether ligand.

The adsorption of different proteins can be promoted by adding different salts to the mixture. Varying the concentration of the loading salt can affect the adsorption affinities of IgG, IgM, IgA, Fab and Fc fragments, and C3 and C4 complement factors. Several sulfate salts can be used to promote the adsorption of target proteins. The most commonly used salts are potassium sulfate, sodium sulfate, and ammonium sulfate. In addition, salt concentration can differentially affect the adsorption kinetics of IgG, IgM, IgA, Fab, and Fc fragments, and complement factors C3 and C4 (3–6).

TAC is an economical technique for purifying immunoglobulins from whole serum and tissue cultures (7, 8). In comparison to Protein A-based immunoadsorbents, thiophilic adsorbents have broader affinity towards immunoglobulins (9). Furthermore, >99% of total proteins are recovered using a thiophilic adsorbent in comparison to less than 92% for phenyl and 75% for octyl agarose adsorbents (10).

Purification of Single-Chain Antibodies

Recombinant, single-chain antibodies are becoming increasingly common in research use because they can be genetically manipulated to bind different proteins and to perform specific, desired functions. However, standard antibody purification methods such as Protein A and Protein G do not work well for single chain antibodies because these antibodies lack the Fc domain that natural antibodies possess. Protein A usually binds to this Fc domain. Because Thiophilic Resin binds to a region other than the Fc domain on single chain antibodies, it is able to purify them (Figure 3).

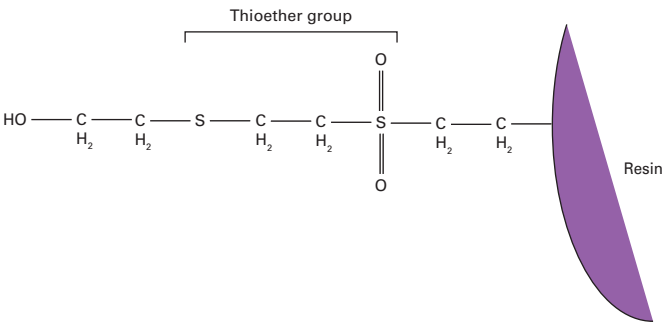

Figure 2. Structure of Thiophilic Resin.

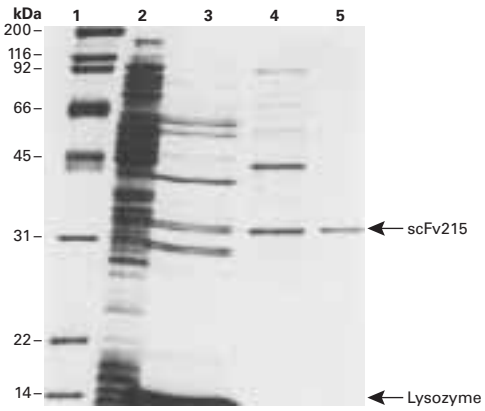

Figure 3. Single-chain antibody purification with Thiophilic Resin. SDS-PAGE analysis of the following samples: bacterial lysate expressing scFv215 (Lane 2), periplasmic fraction (Lane 3), peak fraction from Ni-NTA (Lane 4) and peak fraction from Thiophilic Resin (Lane 5). Shultze, *et al.* 1994 (5). Permission to reprint obtained.

Purification of IgY

Generating antibodies in chickens rather than rabbits is becoming a common method of immunoglobulin production. Antibodies produced in immunized chickens, which are transferred to the egg yolk, can be collected daily from eggs rather than animal serum. Also, chicken egg yolk provides higher amounts of a specific immunoglobulin than rabbit serum (11). Standard immunoglobulin purification methods do not work well for IgY because it does not adsorb to Protein A. In contrast, IgY does adsorb to Thiophilic Resin (Figure 4), which is ideal for this type of purification because it provides a fast, simple, and inexpensive way to obtain large amounts of purified IgY.

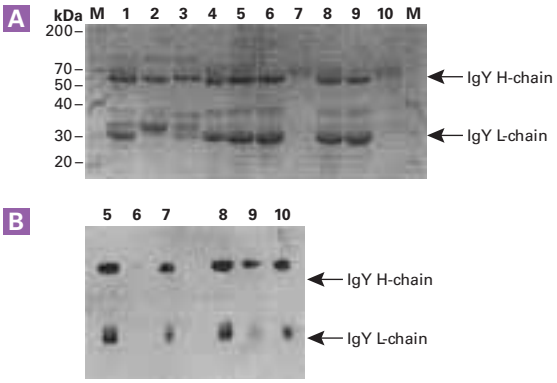

Figure 4. Purification of IgY from chicken egg using Thiophilic Resin. **Panel A.** SDS-PAGE analysis of fractions from purification of chicken egg immunoglobulins. Lanes 7 & 10 each contain 10 mg of protein, and all other lanes, 25 mg each. Lane 1: egg yolk extract supernatant. Lane 2: Supernatant after 60% SAS (Saturated concentration of Ammonium Sulfate). Lane 3: Wash with 60% SAS. Lane 4: Pellet after 60% SAS. Lane 5: column load. Lane 6: unbound material. Lane 7: eluted material. Lanes 8–10: purification with another thiophilic resin. **Panel B.** Immunoblot using 1/10 the amount of material in Panel A. IgY was detected with polyclonal rabbit anti-chicken HRP-conjugate. M=molecular weight. (Hansen et al. 1998 (11); permission to reprint obtained).

Choice of Thiophilic-Superflow and -Uniflow Resins Provides Flexibility

Thiophilic-Superflow and -Uniflow (Table I) can both be used for batch and gravity-flow purification. Thiophilic-Superflow has greater physical strength, making it suitable for FPLC. Thiophilic-Uniflow Resin is prepared using Uniflow 4 agarose cross-linked beads, which permit linear flow rates as high as 2 cm/min. Thiophilic-Superflow Resin is prepared using Superflow 6 agarose crosslinked beads, which permit linear flow rates as high as 5 cm/min. In both cases, the agarose beads were activated with divinylsulfone, and mercaptoethanol was coupled to the activated resin. Both resins can be regenerated and reused without detrimental effects on specificity and capacity.

Table I: Properties of Thiophilic-Uniflow & -Superflow

Feature	Thiophilic-Uniflow	Thiophilic-Superflow
Batch/gravity	Yes	Yes
FPLC	No	Yes
Scale	Analytical	Analytical, preparative
Preparative production capacity (mg IgG/ml adsorbant)	20	25
Matrix	Cross-linked agarose	Cross-linked agarose
Maximum linear flow rate (cm/min)	2.0	5.0
Maximum volumetric flow rate (ml/min) at 5 x 1 cm.i.d	1.6	4.0
pH stability	2–10	2–10
Supplied as	Bulk/slurry 50% in 25% ethanol	Bulk/slurry 50% in 25% ethanol
Storage	4°C, do not freeze	4°C, do not freeze

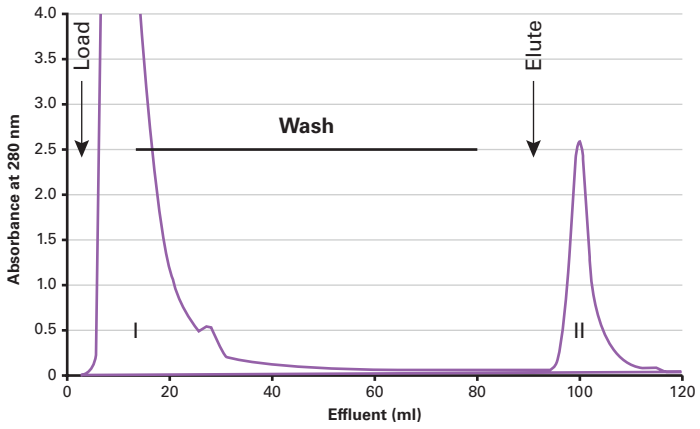

Figure 5. Thiophilic-Superflow Resin purifies IgG at a high flow rate and neutral pH. Whole rabbit serum in 0.5 M sodium sulfate was purified on a Thiophilic-Superflow Resin column and eluted with 0.05 M sodium sulfate (Peak II).

When a Thiophilic-Superflow column was used to purify whole rabbit serum, albumin was effectively removed in the nonadsorbed fraction, allowing the elution of highly purified intact IgG (Figures 5 & 6). Thiophilic adsorbents can also purify other types of proteins such as horseradish peroxidase (12), allergens (13), glutathione peroxidase (14), procollagen (15), acetolactate synthase (16), insect hemolymph proteins (17), serpins (18), lactate dehydrogenase (19), and tuberculosis antigen proteins (20).

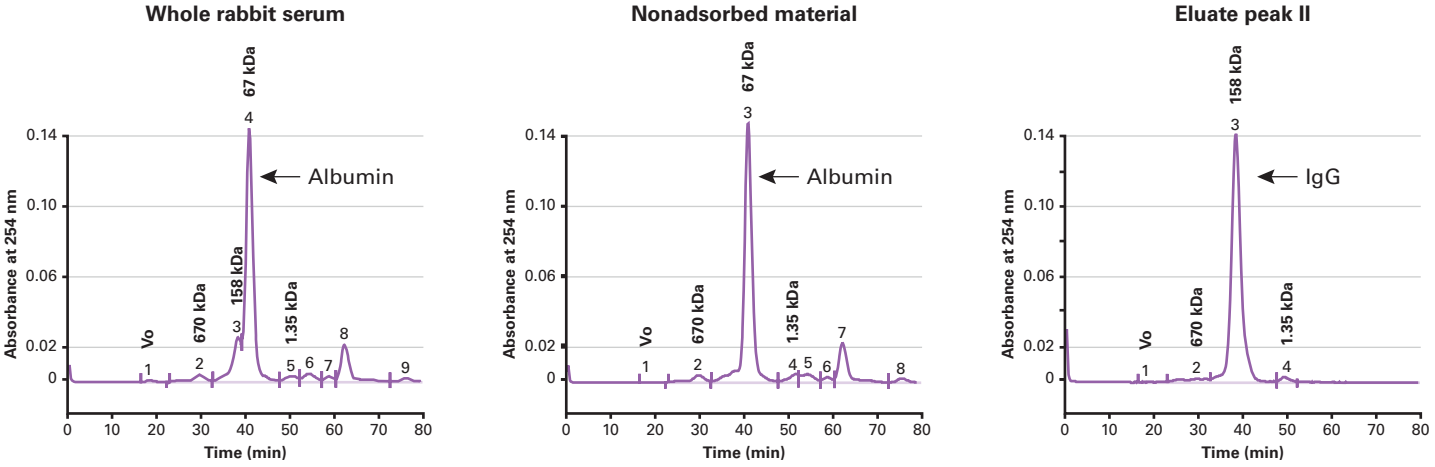

Figure 6. Analysis of purified IgG fractions. Analytical Size Exclusion Chromatography was performed on the purified fractions from Figure 5. Results indicate that the albumin, which constitutes 60–70% of the whole serum, is removed in the nonadsorbed fraction from whole rabbit serum (**Panel A**) and wash (**Panel B**). Then, the intact IgG from Peak II is eluted (**Panel C**).

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Products

Cat. #	Product	Package Size
635616	Thiophilic-Superflow™ Resin	10 ml
635617	Thiophilic-Superflow™ Resin	100 ml
635614	Thiophilic-Uniflow Resin	100 ml