

Purification of immunoglobulins

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. An alternative method of immunoglobulin purification, thiophilic adsorption chromatography, is ideal for these types of applications, as well as immunoglobulin purification in general.

Thiophilic adsorption chromatography (TAC) was developed by Porath *et al.* (1984). TAC is a group-specific, salt-dependent purification technique with distinct adsorption affinity towards immuno-globulins and α_2 -macroglobulins. The term “thiophilic” refers to the affinity that proteins have for sulfone groups that lie in close proximity to thioether groups (Figure 22; Porath *et al.*, 1985).

Advantages of Thiophilic Resin

- Broad selectivity for IgE, IgM, IgY and IgG
- Purify single-chain antibodies
- Reduce the number of purification steps
- High recovery rate
- Purification at neutral pH
- High flow rates of 5 cm/min

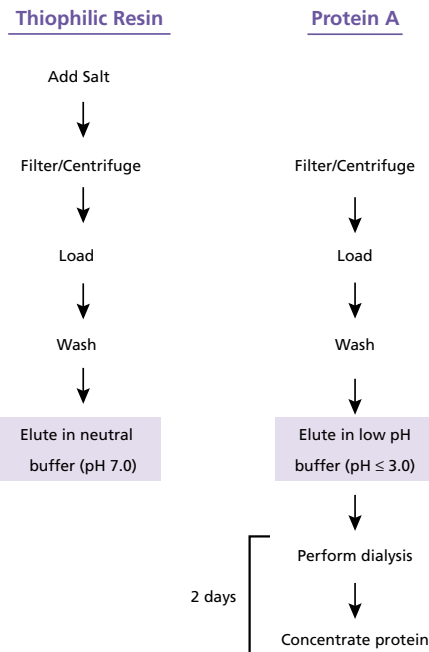


Figure 20. Thiophilic Resin purifies antibodies at neutral pH and more quickly than Protein A.

Purification of immunoglobulins...cont.

Purification of single-chain antibodies

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 (Lutomski *et al.*, 1995; Oscarsson *et al.*, 1992; Schulze *et al.*, 1994; Yurov *et al.*, 1994).

TAC is an economical technique for purifying immunoglobulins from whole serum and tissue cultures (Porath & Belew, 1987; Scoble & Scopes, 1997). In comparison to Protein A-based immunoadsorbents, thiophilic adsorbents have broader affinity towards immunoglobulins (Hutchens & Porath, 1986). 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 (Oscarsson *et al.*, 1995).

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.

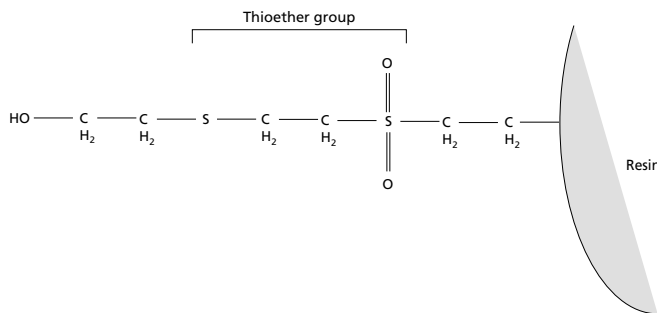


Figure 22. Structure of Thiophilic Resin.

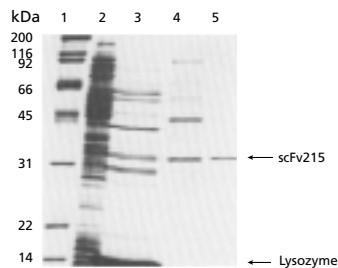


Figure 21. Single-chain antibody purification with Thiophilic Resin. SDS-PAGE analysis of the following samples: Bacterial lysate expressing scFv215 (lane 2), Periplasmic fraction (lane 3), peak fraction from Ni-NTA (lane 4) and peak fraction from thiophilic resin (lane 5). Shultz, *et al.* 1994. Permission to reprint obtained.

Purification of immunoglobulins...cont.

Purification of IgY

Generating antibodies in chickens rather than rabbits is becoming a common method of immunoglobulin production. Antibodies produced in immunized chickens are transferred to the egg yolk, so antibodies can be collected daily from eggs rather than animal serum. Also, higher amounts of a specific immunoglobulin can be obtained from chicken egg yolk than from rabbit serum (Hansen *et al.*, 1988). Standard immunoglobulin purification methods do not work well for purifying IgY because IgY does not adsorb to Protein A. In contrast, IgY does adsorb to Thiophilic Resin. Our Thiophilic Resin is ideal for this type of purification because it provides a fast, simple, and inexpensive way to obtain large amounts of purified IgY.

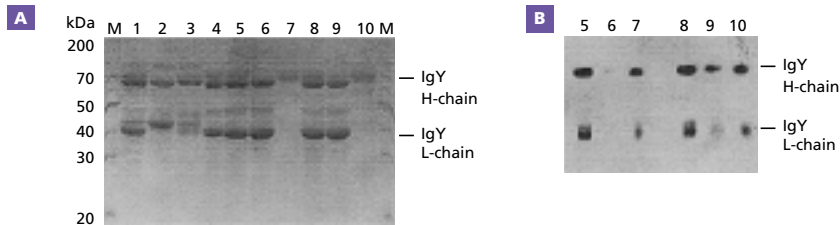


Figure 23. Purification of IgY from chicken egg using Thiophilic Resin. Panel A. SDS-PAGE analysis of fractions from purification of chicken egg immunoglobulins. In lanes 7 & 10, 10 mg of protein was loaded, and all other lanes, 25 mg was loaded. Lane 1: supernatant of egg yolk extract. Lane 2: supernatant 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 using another type of thiophilic resin. **Panel B.** Immunoblot of Panel A results. One tenth of the material of the gel in panel A was loaded, then immunoblotted. The IgY was detected with polyclonal rabbit anti-chicken HRP-conjugate. M=molecular weight. (Hansen *et al.* 1998; permission to reprint obtained).

Purification of Immunoglobulins...cont.

Thiophilic-Superflow and -Uniflow can both be used for batch and gravity-flow purification. Thiophilic-Superflow has greater physical strength, which makes it suitable for FPLC. Table VII (page 32) compares the features of the two different resins.

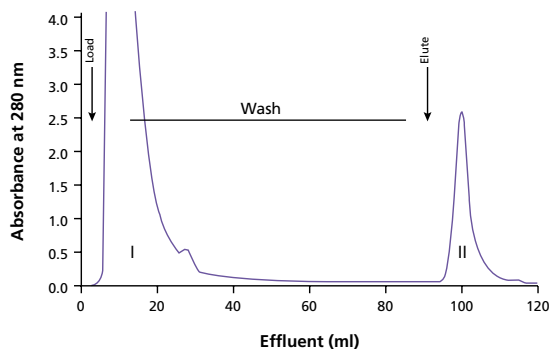


Figure 24. Thiophilic-Superflow Resin purifies IgG at high flow rate and neutral pH. Whole rabbit serum in 0.5 M sodium sulfate was purified on a Thiophilic-Superflow Resin column and eluted with 0.05 M sodium sulfate (peak II).

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 cross-linked 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 Thiophilic-Uniflow and -Superflow can be regenerated and reused without detrimental effects on specificity and capacity.

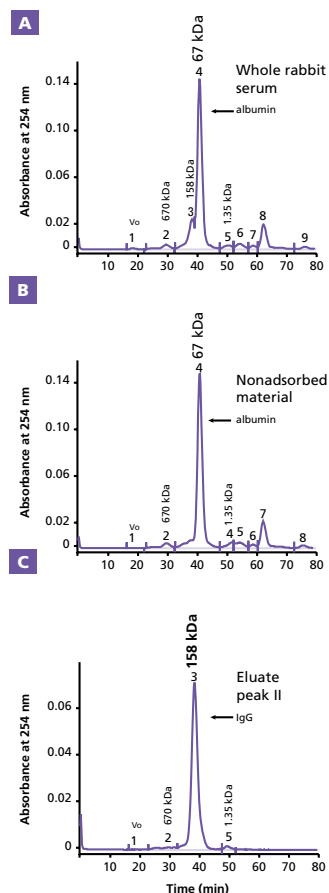


Figure 25. Analysis of purified IgG fractions. Analytical Size Exclusion Chromatography was performed on the purified fractions from Figure 24. Results indicate that the albumin, which constitutes 60–70% of the whole serum, is removed in the non-adsorbed fraction from whole rabbit serum (Panel A) and wash (Panel B). Then, the intact IgG from Peak II is eluted (Panel C).

Purification of immunoglobulins...*cont.*

Table VII: 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 adsorbent)	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

Thiophilic adsorbents can also purify other types of proteins such as horseradish peroxidase (Chaga *et al.*, 1992), allergens (Goubran-Botros *et al.*, 1998), glutathione peroxidase (Huang *et al.*, 1994), procollagen (Pedersen & Bonde, 1994), acetolactate synthase (Poulsen & Stougaard, 1989), insect hemolymph proteins (Samaraweera *et al.*, 1992), serpins (Rosenkrands *et al.*, 1994), lactate dehydrogenase (Kminkova & Kucera, 1998), and tuberculosis antigen proteins (Rosenkrands *et al.*, 1998).

TALON Product List	Size	Cat. #
TALON Metal Affinity Resin	10 ml	8901-1
	25 ml	8901-2
	100 ml	8901-3
	250 ml	8901-4
TALONspin Columns	10 cols.	8902-1
	25 cols.	8902-2
	50 cols.	8902-3
	100 cols.	8902-4
TALON 2-ml Disposable Gravity Column	50 cols.	8903-1
TALON Superflow Metal Affinity Resin	25 ml	8908-1
	100 ml	8908-2
TALON CellThru	10 ml	8910-1
	100 ml	8910-2
CellThru 2-ml Disposable Columns	50 columns	8914-1
CellThru 10-ml Disposable Columns	20 columns	8915-1
TALON Buffer Kit	each	K1252-1
TALON Purification Kit	each	K1253-1
Talon-Dextran Trial Size	5 mg	8918-y

Thiophilic Resin Product List

Thiophilic-Uniflow Resin	10 ml	8913-1
	100 ml	8913-2
Thiophilic-Superflow Resin	10 ml	8917-1
	100 ml	8917-2

Glutathione Resin Product List

Glutathione-Superflow Resin	10 ml	8911-1
	100 ml	8911-2
Glutathione-Uniflow Resin	10 ml	8912-1
	100 ml	8912-2
GST Purification Kit	5 purifications	K1251-1