

Simplified manual purification of antibodies in 30 min

In antibody purification, speed and simplicity are important factors to minimize protein instability and degradation. With rProtein A, Protein G, and rProtein A/Protein G GraviTrap™ prepacked gravity-flow columns, up to 50 mg of antibodies from cell cultures and biological fluids can be purified in less than 30 min. The prepacked format ensures high consistency between experiments and save times since there is no need to pack the column. The product packaging converts to a convenient lab stand and there is no need for instruments or other complex equipment to perform the purification.

Simple four-step procedure

Manual purification of immunoglobulins with GraviTrap columns is achieved with a simple four-step procedure (Fig 1). A wide choice of buffers is available since these media have high affinities for a broad range of immunoglobulins at about pH 7.0. To attain effective binding, the pH of the sample should be the same as that of the binding buffer prior to sample application.

Reliable binding and easy elution

The binding strengths of protein A and protein G for antibodies depend on the source species and subclass of the particular IgG. GE Healthcare therefore provides GraviTrap columns prepacked with three alternative Sepharose™ based column media: rProtein A, Protein G, and a mixture, rProtein A/Protein G. Both rProtein A and Protein G media give reliable purification of antibodies through fast kinetics and high binding capacity (20 to 50 mg/ml). The purity achieved is often > 95%, with recoveries of typically 70% to 80%. GraviTrap columns for antibodies are reusable up to five times.

IgG elution is achieved by lowering the pH. Different immunoglobulins elute at different pH values depending on the subclass and the species from which they originate. Therefore, addition of a neutralizing buffer to the eluate is recommended to preserve the activity of acid-labile IgG.

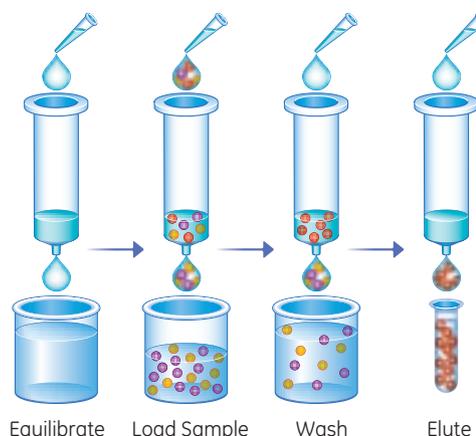


Fig 1. Manual four-step procedure for purification of antibodies using GraviTrap columns: Equilibration, sample application, washing, and elution.

Applications

rProtein A/Protein G GraviTrap columns were used to purify human monoclonal antibodies from a CHO cell cultivation media. The total purification time was 26 min with a recovery of 67%, and the purity was high as determined by SDS-PAGE (Fig 2A).

Similarly, two rProtein A GraviTrap columns were used to purify rabbit anti-goat IgG from serum. The purification time was 21 min, and the purity was high as determined by SDS-PAGE (Fig 2B). Approximately 11 mg of purified rabbit IgG was obtained on each column in a single run.

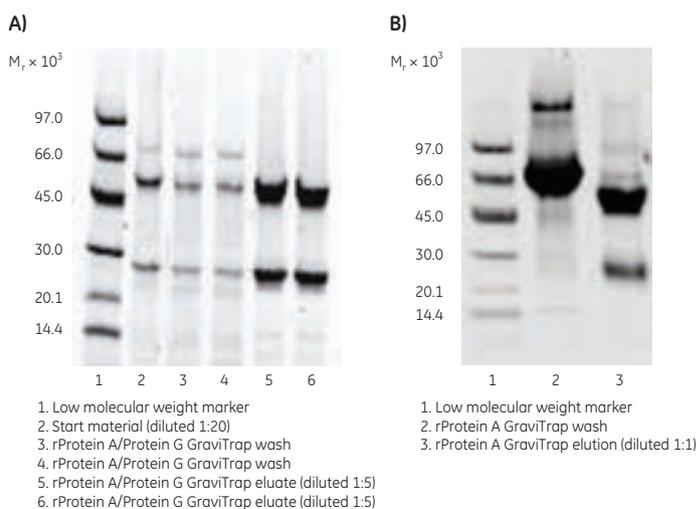


Fig 2. Deep Purple™ stained SDS-PAGE analysis (reduced conditions) of fractions from **A)** purification of human monoclonal antibodies on rProtein A/Protein G GraviTrap; **B)** eluted fractions from purification of rabbit serum on rProtein A GraviTrap.

Ordering information

Product	Code number
rProtein A GraviTrap, 10 × 1 ml	28-9852-54
Protein G GraviTrap, 10 × 1 ml	28-9852-55
rProtein A/Protein G GraviTrap, 10 × 1 ml	28-9852-56

For more information, visit www.gelifesciences.com/sampleprep

