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Product Information

Protein A Antibody Purification Kit

Catalog Number **PURE1A**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Protein A Antibody Purification Kit provides all of the reagents necessary to isolate monoclonal and polyclonal antibodies in an easy-to-use format. The reagents provided are sufficient for 10 affinity chromatographic procedures. The kit is suitable for use with IgGs from the following species: human, mouse, rabbit, goat, and bovine. The kit may also be applicable for the purification of IgG from other species; however, it is up to the individual researcher to determine the suitability for their particular use.

The kit produces purified antibody, at a physiological pH, ready for use in other procedures, without the deleterious effects of prolonged exposure to low pH conditions.

Each Protein A cartridge is capable of purifying the following quantities of IgG during each use:

Species	IgG (mg)
Human	20-30
Mouse	6-12
Rabbit	15-20
Goat	6-12
Bovine	15-20

Materials Provided

- Protein A Cartridge 1 mL
- Desalting Cartridge 1 each
- Binding Buffer 225 mL
- Elution Buffer 75 mL
- Regeneration Buffer 75 mL
- HEPES Buffer 225 mL
- Plastic syringe 5 mL
- Plastic syringe 10 mL

Materials required but not provided

- 0.45 µm filters (Catalog Number CLS431220)
- 12 × 75 mm test tubes
- 50 mL beakers
- Phosphate Buffered Saline containing 0.02% sodium azide
  PBS Tablets (Catalog Number P4417)
  Sodium azide (Catalog Number S2002)

Materials suggested but not provided

- Additional 5 and 10 mL syringes
- Timer
- Clamp and ring stand

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Due to the sodium azide content of the kit buffers, a material safety data sheet (MSDS) has been sent to the attention of the safety officer of your institution. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store the kit at 2–8 °C. **Do Not Freeze.**

For long term storage of the cartridges, wash the cartridges with 10 mL of phosphate buffered saline containing 0.02% sodium azide. Cap the cartridges with the end closures provided and store at 2–8 °C. **Do Not Freeze.**

Procedure

Clarified serum, ascites fluid, or culture supernatant is first loaded onto the Protein A Cartridge where the IgG is immobilized. The Protein A Cartridge is then washed to remove excess unbound proteins. The Desalting Cartridge is readied for use by reactivating with HEPES buffer. The Protein A Cartridge and Desalting Cartridge are then connected via the Luer lock fittings and the Elution Buffer is introduced. The eluate contains the purified IgG at a physiological pH. Both cartridges may be regenerated and stored for future use.

Sample Preparation

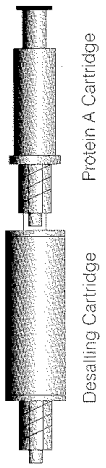
Serum or ascites samples must be clarified by centrifugation or filtration with a 0.45 μm filter prior to use with the Protein A Cartridge. Hybridoma culture supernatants may be used directly if they are visibly clear, otherwise clarify by centrifugation of filtration.

1. Serum or Ascites samples:
   a. Estimate the IgG content of the sample if possible. The Protein A Cartridge will bind approximately the IgG content contained in 2 mL of human serum or ~2 mL of mouse ascites fluid containing 5 mg/mL IgG.
   b. To each 2 mL of serum or ascites fluid, add 4 mL of Binding Buffer and mix well.
2. Hybridoma Culture Supernatants:
   a. Estimate the IgG content of the sample if possible. The Protein A Cartridge can bind up to 10 mg of monoclonal antibody.
   b. To each 10 mL of culture supernatant, add 1 ml of Binding Buffer and mix well.

Sample Purification

Note: The cartridge is designed to be used in the vertical position during loading, washing, and eluting operations. Care should be taken to avoid the introduction of air bubbles into the cartridge. Air bubbles decrease the separation efficiency and increase the pressure required to generate adequate flow through the cartridge. Bubbles can be removed from the cartridge by gentle tapping.

1. Fill the 10 mL syringe with 10 mL of HEPES Buffer and connect to the top (male connector) of the Desalting Cartridge. Wash the cartridge by passing the buffer through the cartridge at an approximate flow rate of 1 mL/minute.
2. Fill the 5 mL syringe with 5 mL of Regeneration Buffer and connect it to the top (male connector) of the Protein A Cartridge. Wash the cartridge by passing the Regeneration Buffer through the cartridge at an approximate flow rate of 1 mL/minute.
3. Fill the 10 mL syringe with 4 mL of Binding Buffer. Equilibrate the Protein A Cartridge by passing the Binding Buffer through the cartridge at an approximate flow rate of 1 mL/minute.
4. Fill the 10 mL syringe with the sample-Binding Buffer mixture. Load the Protein A Cartridge by passing the sample-Binding Buffer mixture through the cartridge at an approximate flow rate of 0.5 mL/minute.
5. After the sample is loaded onto the Protein A Cartridge, rinse the 10 mL syringe with water, refill it with 6 mL of Binding Buffer and wash the Protein A Cartridge by passing the buffer through the cartridge at an approximate flow rate of 1 mL/minute.
6. Attach the female end of the Protein A Cartridge to the male Luer lock fitting of the Desalting Cartridge. See figure.



7. Fill the 5 mL syringe with 5 mL of Elution Buffer. Attach the syringe to the Protein A Cartridge. Elute the cartridges by passing the Elution Buffer through the cartridges at an approximate flow rate of 0.5 mL/minute. The eluate contains the purified immunoglobulin at a physiological pH.
8. Detach the two cartridges.
   a. Regenerate the Protein A Cartridge by passing 5 mL of Regeneration Buffer through the cartridge.
   b. Regenerate the Desalting Cartridge by passing 10 mL of HEPES Buffer through the cartridge.

   The cartridges are now ready for another affinity chromatographic purification.
9. For long term storage of the cartridges, wash the cartridges with 10 mL of phosphate buffered saline containing 0.02% sodium azide. Cap the cartridges with the end closures provided and store at 2–8 °C. **Do Not Freeze.**

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