Ab Buffer Kit

Ab Buffer Kit contains 10× stock solutions of Binding and Elution buffers and 1× Neutralizing buffer optimized for rapid purification of monoclonal and polyclonal IgG using immobilized Protein A or Protein G.

The kit eliminates time-consuming buffer preparation and thus promotes fast, reproducible, and convenient purification work.

The buffers have been prepared using the highest quality chemicals and water, and have been filtered through a 0.45 µm filter.

Basic purification protocols

The basic protocols outlined below describe two common procedures where buffers prepared with Ab Buffer Kit can be used.

In the first, 1-ml or 5-ml HiTrap™ columns prepacked with either Protein A or Protein G Sepharose™ media are used. In the second protocol, Ab SpinTrap™, a prepacked single-use spin column is used.

When performing any of these procedures, also check the relevant column instructions.

Tables 1, 2, and 3 provide useful information for mixing buffers for use in the protocols provided below.

Note: Neutralizing buffer does not need dilution

Buffer mixing tables

Table 1. Buffer preparation for *one* purification on a HiTrap Protein G HP, HiTrap Protein A HP, or HiTrap rProtein A FF 1 ml column using a syringe

	10× Buffer concentrate	Distilled water	Final volume
Binding buffer	2.5 ml	22.5 ml	25 ml
Elution buffer	0.5 ml	4.5 ml	5 ml

Table 2. Buffer preparation for *one* purification on a HiTrap Protein G HP, HiTrap Protein A HP or HiTrap rProtein A FF 5 ml column using a syringe

	10× Buffer concentrate	Distilled water	Final volume
Binding buffer	12.5 ml	112.5 ml	125 ml
Elution buffer	2.5 ml	22.5 ml	25 ml

Table 3. Buffer preparation for *ten* purifications on Ab SpinTrap columns

	10× Buffer concentrate	Distilled water	Final volume
Binding buffer	2 ml	18 ml	20 ml
Elution buffer	1 ml	9 ml	10 ml

Purification on HiTrap Protein G HP, HiTrap Protein A HP, and HiTrap rProtein A FF (1 ml or 5 ml) using a syringe

Recommended flow rates: approx. 1-3 ml/min for HiTrap 1 ml columns; approx. 5-10 ml/min for HiTrap 5 ml columns. Prepare buffers according to Table 1 or 2.

Prepare collection tubes by adding 60 to 200 µl of Neutralizing buffer per ml of fraction to be collected. In this way the final pH of the eluted antibodies will be approximately neutral, which preserves the activity of acid labile IgG.

1. Prepare the sample by centrifugation (10 000 × g, 20 min) or filter (0.45 µm) if there are particles present or the appearance is cloudy. If the sample is serum or ascites fluid, dilute the sample 1:1 with Binding buffer.
2. Fill the syringe with Binding buffer. Remove the stopper and connect the column to the syringe (with the provided adapter) "drop to drop" to avoid introducing air into the column.
3. Remove the snap-off end at the column outlet.
4. Wash the column with 10 column volumes (CV) of Binding buffer (1 CV = 1 ml for HiTrap 1-ml column; 1 CV = 5 ml for HiTrap 5-ml column).
5. Apply the sample, using a syringe fitted to the Luer adapter. Collect the flowthrough.
6. Wash with 5 to 10 CV of Binding buffer or until no material appears in the effluent. Collect wash fractions.
7. Elute with 2 to 5 CV of Elution buffer. Collect 1-ml fractions in collection tubes containing Neutralizing buffer. Purified IgG will most likely be found in the second and/or third fractions.

Purification on Ab SpinTrap

This protocol is optimized for performance at room temperature. If performed at a colder temperature, a longer incubation time may be needed.

Use Ab SpinTrap columns with a standard microcentrifuge. Place a column in a 2-ml microcentrifuge tube to collect liquid during centrifugation. Prepare buffers according to Table 3.

Prepare two collection tubes per sample for eluted fractions by adding 30 µl Neutralizing buffer to a 2-ml microcentrifuge tube.

1. Invert and shake the Ab SpinTrap column repeatedly to resuspend the medium. Loosen the top cap one-quarter of a turn and break off the bottom closure. Place the column in a 2-ml microcentrifuge tube and centrifuge for 30 s at 70 to 100 × g to remove the storage liquid (approx. 1000 rpm in an Eppendorf™ 5415R, 24-position fixed-angle rotor).
2. Remove the top cap. Equilibrate the column by adding 600 µl Binding buffer. Centrifuge for 30 s at 70 to 100 × g.
3. Add the sample. Maximum sample volume is 600 µl/load. Secure the top cap tightly and incubate for 4 min with gentle mixing (mixing with an end-over-end mixer machine is recommended but other methods can also be used). Loosen the top cap one-quarter of a turn, centrifuge for 30 s at 70 to 100 × g. Several sample applications can be performed provided that the capacity of the column is not exceeded. Repeat this step after each application. Empty the microcentrifuge tube as needed.
4. Wash with 600 µl Binding buffer. Centrifuge for 30 s at 70 to 100 × g. Repeat the wash.
5. Elute the bound antibody by adding 400 µl Elution buffer to the column. Place the column in a 2-ml microcentrifuge tube containing 30 µl Neutralizing buffer. Centrifuge for 30 s at 70 × g. The eluted fraction contains the purified antibody. Repeat this step one time, collecting the second elution in a fresh 2-ml microcentrifuge tube containing 30 µl Neutralizing buffer (most of the bound antibody is eluted after two elution steps).

Ordering information

Description	Quantity	Code No.
Ab Buffer Kit	1	28-9030-59
(Includes Binding buffer 10× concentrate, 50 ml; Elution buffer 10× concentrate, 15 ml; Neutralizing buffer, 25 ml)		

Related products

Ab SpinTrap	50 × 100 µl	28-4083-47
HiTrap Protein G HP, 1 ml	2 × 1 ml	17-0404-03
HiTrap Protein G HP, 1ml	5 × 1 ml	17-0404-01
HiTrap Protein G HP, 5 ml	1 × 5 ml	17-0405-01
HiTrap Protein G HP, 5 ml	5 × 5 ml	17-0405-03
HiTrap Protein A HP, 1 ml	2 × 1 ml	17-0402-03
HiTrap Protein A HP, 1ml	5 × 1 ml	17-0402-01
HiTrap Protein A HP, 5 ml	1 × 5 ml	17-0403-01
HiTrap Protein A HP, 5 ml	5 × 5 ml	17-0403-03
HiTrap rProtein A FF, 1 ml	2 × 1 ml	17-5079-02
HiTrap rProtein A FF, 1 ml	5 × 1 ml	17-5079-01
HiTrap rProtein A FF, 1 ml	1 × 5 ml	17-5080-01
HiTrap rProtein A FF, 1 ml	5 × 5 ml	17-5080-02

Literature

Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86

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