Ab Buffer Kit

Introduction

Ab Buffer Kit contains 10X stock solutions of Binding and Elution buffers and ready to use Neutralizing buffer. The buffers are 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.

The following protocols can be used with buffers from Ab Buffer Kit:

- Protocol 1, using 1 ml or 5 ml HiTrap™ columns.
- Protocol 2, using SpinTrap™ columns (Protein A HP SpinTrap, Protein G HP SpinTrap and Ab SpinTrap).
- For Ab purification using multiwell plates, please follow the instruction provided with the Protein A HP or Protein G HP MultiTrap™ product.

Kit content

Buffer	Buffer content	Formulation	Volume
Binding Buffer	0.2 M sodium phosphate, pH 7.0*	10X	50 ml
Elution Buffer	1 M glycine-HCl, pH 2.7	10X	15 ml
Neutralizing buffer	1 M Tris-HCl, pH 9*	Ready to use	25 ml

* Containing 20% ethanol as a preservative

Protocol 1

Use this protocol for:

- HiTrap Protein G HP
- HiTrap Protein A HP
- HiTrap Protein A FF

Recommendations

- 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.
- Check also the relevant column instructions.

Buffer preparation.

Table 1. Buffer preparation for 1 purification on 1 ml HiTrap columns.

Buffer	Stock solution	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 1 purification on 5 ml HiTrap columns.

Buffer	Stock solution	Distilled water	Final volume
Binding buffer	12.5 ml	112.5 ml	125 ml
Elution buffer	2.5 ml	22.5 ml	25 ml

Protocol

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.

Protocol 2

Use this protocol for:

- Ab SpinTrap
- Protein A HP SpinTrap
- Protein G HP SpinTrap

Recommendations

- The protocol is optimized for room temperature. If performed at a lower 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.
- Check also the relevant column instructions.

Buffer preparation

Table 3. Buffer preparation for 10 purifications on Ab SpinTrap columns or 20 reactions using MultiTrap multiwell plates.

Buffer	Stock solution	Distilled water	Final volume
Binding buffer	2 ml	18 ml	20 ml
Elution buffer	1 ml	9 ml	10 ml

Protocol

1 Invert and shake the Ab SpinTrap column repeatedly to resuspend the medium.

2 Loosen the top cap one-quarter of a turn and break off the bottom closure.

3 Place the column in a 2-ml microcentrifuge tube and centrifuge for 30 s at 70 to 100 × *g* to remove the storage liquid.

4 Remove the top cap. Equilibrate the column by adding 600 µl Binding buffer. Centrifuge for 30 s at 70 to 100 × *g*.

5 Add the sample. Maximum sample volume is 600 µl/load.

6 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).

7 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.

8 Wash with 600 µl Binding buffer. Centrifuge for 30 s at 70 to 100 × *g*.

9 Repeat the wash.

10 Elute the bound antibody by adding 400 µl Elution buffer to the column.

11 Place the column in a 2-ml microcentrifuge tube containing 30 µl Neutralizing buffer.

12 Centrifuge for 30 s at 70 × *g*.
The eluted fraction contains the purified antibody.

13 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

Products

Description	Quantity	Code No.
Ab Buffer Kit	1	28-9030-59

Related products

Description	Quantity	Code No.
Protein A HP SpinTrap	16 columns	28-9031-32
Protein G HP SpinTrap	16 columns	28-9031-34
Ab SpinTrap	50 columns	28-4083-97
Protein A HP MultiTrap	4 × 96-well plates	28-9091-33
Protein G HP MultiTrap	4 × 96-well plates	28-9091-35
HiTrap Protein G HP, 1 ml	2 columns	17-0404-03
HiTrap Protein G HP, 1 ml	5 columns	17-0404-01
HiTrap Protein G HP, 5 ml	1 column	17-0405-01
HiTrap Protein G HP, 5 ml	5 columns	17-0405-03
HiTrap Protein A HP, 1 ml	2 columns	17-0402-03
HiTrap Protein A HP, 1 ml	5 columns	17-0402-01
HiTrap Protein A HP, 5 ml	1 column	17-0403-01
HiTrap Protein A HP, 5 ml	5 columns	17-0403-03
HiTrap rProtein A FF, 1 ml	2 columns	17-5079-02
HiTrap rProtein A FF, 1 ml	5 columns	17-5079-01
HiTrap rProtein A FF, 5 ml	1 column	17-5080-01
HiTrap rProtein A FF, 5 ml	5 columns	17-5080-02

Literature

Title	Code No.
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook	18-1022-29
Affinity Chromatography Columns and Media Product Profile	18-1121-86

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