INSTRUCTIONS

# Gentle Ag/Ab Binding and Elution Buffers

21012 21020 21013 21027 21030 0120.3

| Number | Description |
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| **21012** | **Gentle Ag/Ab Binding Buffer,** pH 8.0, 3.75L |
| **21020** | **Gentle Ag/Ab Binding Buffer,** pH 8.0, 1L |
| **21013** | **Gentle Ag/Ab Elution Buffer,** pH 6.6, 3.75L |
| **21027** | **Gentle Ag/Ab Elution Buffer,** pH 6.6, 500mL |
| **21030** | **Gentle Ag/Ab Binding and Elution Buffer Kit** |

**Kit Contents:**

**Gentle Ag/Ab Binding Buffer,** pH 8.0, 100mL

**Gentle Ag/Ab Elution Buffer,** pH 6.6, 100mL

**Storage:** Upon receipt store at 4°C. Products shipped at ambient temperature.

## Introduction

Thermo Scientific Gentle Ag/Ab Elution Buffer is for dissociation of antigen/antibody (Ag/Ab) interactions without harsh or permanently denaturing components. Unlike typical elution buffers that involve potentially damaging acidic or alkaline conditions, the Gentle Ag/Ab Elution Buffer is a near-neutral (pH 6.55), high-salt solution for effectively dissociating affinity interactions while preserving both antibody and antigen activities.

Thermo Scientific Gentle Ag/Ab Binding Buffer is specifically formulated for use with Immobilized Protein A, whose optimal antibody-binding condition is pH 8.0. The Gentle Binding Buffer must be used with the Gentle Elution Buffer in this particular affinity system because the regular IgG (Protein A) Binding Buffer (see Related Thermo Scientific Products) contains phosphate ions that form an insoluble precipitate upon contact with the Gentle Elution Buffer salts. The Gentle Binding Buffer may not be optimal for interactions other than Protein A binding.

## Important Product Information

Avoid using binding and wash buffers that contain phosphate ions (e.g., phosphate-buffered saline, PBS) because these will cause precipitation in the sample when the Gentle Elution Buffer is applied. Use a non-phosphate binding and wash buffer that has the appropriate pH and ionic strength for the affinity interaction being used. Typical Ag/Ab binding interactions occur optimally at physiological conditions (pH 7.2-7.4, 150mM salt), and a solution with these parameters can be made with Tris, HEPES, MOPS or another non-phosphate buffer. For more detailed suggestions for particular applications, refer to the following example procedures.

### Gentle Antibody Purification with Immobilized Protein A or Protein A/G

**Note:** Do not use IgG (A) Binding Buffer or IgG (A/G) Binding Buffer if the Gentle Ag/Ab Elution Buffer will be used. For a binding buffer, use either the Gentle Ag/Ab Binding Buffer (optimal) or a phosphate-free buffer such as Tris-buffered saline (TBS, e.g., Product No. 28379).

1. Equilibrate buffers and column of Immobilized Protein A to the same temperature (e.g., room temperature or 4°C).

2. Prepare antibody sample for binding. Dilute concentrated samples such as serum and ascites fluid with an equal volume of Gentle Ag/Ab Binding Buffer. Adjust cell culture supernatant and other dilute samples to pH 7.5-8.0 with phosphate-free buffer. Centrifuge cloudy samples and use only the clear supernatant.
3. Wash and prepare the Protein A column by adding five gel-bed volumes of Gentle Ag/Ab Binding Buffer and allowing it to flow through. Discard the flow-through storage buffer.
4. Add the prepared antibody sample to the Immobilized Protein A column and allow it to flow through. If desired, stop the column occasionally or control the flow rate to ensure adequate binding time. Save the flow-through non-bound sample.
5. Wash column by adding 5-10 gel-bed volumes of Gentle Ag/Ab Binding Buffer or other phosphate-free buffer and allowing it to flow through. If desired, analyze flow-through fractions to determine if washing is complete.
6. Elute the purified antibody by adding 5-10 gel-bed volumes of Gentle Ag/Ab Elution Buffer and collecting the flow-through in several small fractions. After step 7, when fractions can be analyzed, those confirmed to contain antibody may be pooled together, and the other fractions may be discarded.
7. Dialyze or use a desalting column to exchange the purified antibody fractions into a phosphate-free buffer for storage and analysis.

   **Notes:**
   - Do not buffer-exchange directly into phosphate buffer because precipitation will occur.
   - Do not mix eluted antibody sample directly with SDS-PAGE sample loading buffer because precipitation will result and adversely affect electrophoresis.

## Gentle Antibody Elution from Immobilized Protein G

**Note:** Do not use a binding/wash buffer that contains phosphate ions. For optimal results, use IgG (G) Binding Buffer for both binding and wash steps. Alternatively, use a phosphate-free binding/wash buffer such as Tris-buffered saline (TBS, e.g., Product No. 28379).

1. Equilibrate buffers and column of Immobilized Protein G to the same temperature (e.g., room temperature or 4°C).
2. Prepare antibody sample for binding. Dilute concentrated samples such as serum and ascites fluid with an equal volume of IgG (G) Binding Buffer. Adjust cell culture supernatant and other dilute samples to pH 6-7 with phosphate-free buffer. Centrifuge cloudy samples and use only the clear supernatant.
3. Wash and prepare the Protein G column by adding five gel-bed volumes of IgG (G) Binding Buffer and allowing it to flow through. Discard the flow-through storage buffer.
4. Add the prepared antibody sample to the Immobilized Protein G column and allow it to flow through. If desired, stop the column occasionally or control the flow rate to ensure adequate binding time. Save the flow-through non-bound sample.
5. Wash column by adding 5-10 gel-bed volumes of IgG (G) Binding Buffer or other phosphate-free buffer and allowing it to flow through. If desired, analyze flow-through fractions to determine if washing is complete.
6. Elute the purified antibody by adding 5-10 gel-bed volumes of Gentle Ag/Ab Elution Buffer and collecting the flow-through in several small fractions. After the step 7, when fractions can be analyzed, those confirmed to contain antibody may be pooled together, and the other fractions may be discarded.
7. Dialyze or use a desalting column to exchange the purified antibody fractions into a phosphate-free buffer for storage and analysis.

   **Notes:**
   - Do not buffer-exchange directly into phosphate buffer because precipitation will occur.
   - Do not mix eluted antibody sample directly with SDS-PAGE sample loading buffer because precipitation will result and adversely affect electrophoresis.

## Gentle Elution of Antibody/Antigen Interactions

**Notes:**

- Do not use a wash buffer that contains phosphate ions. For optimal results, use a phosphate-free binding/wash buffer such as Tris-buffered saline (TBS, e.g., Product No. 28379) or HEPES buffer. Gentle Ag/Ab Binding Buffer, which is a borate buffer at pH 8.0, may not be optimal for the Ag/Ab binding interaction.
- This example procedure assumes that an antigen (e.g., peptide) has been immobilized to a beaded agarose resin and is being used in a column format to purify antibody from a serum or culture supernatant sample. Use the same procedure for the converse purification scheme (i.e., immobilized antibody column to purify an antigen) by simply switching all references to antigen and antibody.

1. Equilibrate buffers and column of immobilized antigen to the same temperature (room temperature or 4°C).
2. Prepare antibody sample for binding. Dilute concentrated samples such as serum and ascites fluid with an equal volume of TBS or HEPES buffer, pH 7.2-7.4. Adjust cell culture supernatant and other dilute samples to pH 7.2-7.4 with phosphate-free buffer. Centrifuge cloudy samples and use only the clear supernatant.
3. Wash and prepare the antigen column by adding five gel-bed volumes of binding buffer and allowing it to flow through. Discard the flow-through storage buffer.
4. Add the prepared antibody sample to the antigen column and allow it to flow through. If desired, stop the column occasionally or control the flow rate to ensure adequate binding time. Save the flow-through non-bound sample.
5. Wash column by adding 5-10 gel-bed volumes of phosphate-free buffer and allowing it to flow through. If desired, analyze flow-through fractions to determine if washing is complete.
6. Elute the purified antibody by adding 5-10 gel-bed volumes of Gentle Ag/Ab Elution Buffer and collecting the flow-through in several small fractions. After the step 7, when fractions can be analyzed, those confirmed to contain antibody may be pooled together, and the other fractions may be discarded.
7. Dialyze or desalting to exchange the purified antibody fractions into a phosphate-free buffer for storage and analysis.

   **Note:** Do not buffer-exchange directly into phosphate buffer because precipitation will occur. Do not mix eluted antibody sample directly with SDS-PAGE sample loading buffer because precipitation will result and adversely affect electrophoresis.

## Gentle Elution of Immunoprecipitation (IP) Reactions

**Notes:**

- Do not use a wash buffer that contains phosphate ions. For optimal results, use a phosphate-free binding/wash buffer at pH 7.2-7.4, such as Tris-buffered saline (TBS, e.g., Product No. 28379) or HEPES buffer.
- This example procedure assumes that 200µL of settled agarose beads are being used in a spin-cup format. For different amounts of affinity beads, scale wash and elution volumes accordingly.

1. Wash and equilibrate the prepared Protein A, Protein G or immobilized antibody affinity gel (hereafter referred to as the Affinity Gel) in the binding buffer that is recommended by the kit procedure (e.g., phosphate-buffered saline, PBS) or an alternative phosphate-free binding/wash buffer.
2. Add the prepared immunoprecipitation sample to the prepared Affinity Gel, and incubate with mixing for 1 hour to overnight, as recommended in the kit instructions.
3. Wash Affinity Gel at least four times with a phosphate-free binding/wash buffer. Do not use PBS or any other type of phosphate buffer for this step.
4. Elute the antigen with 4 × 190µL aliquots of Gentle Ag/Ab Elution Buffer (i.e., Add 190µL Elution Buffer, mix with Affinity Gel, centrifuge, recover solution; Repeat 3 additional times).
5. Dialyze or use a desalting column to exchange the IP fractions into phosphate-free buffer for storage and analysis.

   **Note:** Do not mix eluted antibody sample directly with SDS-PAGE sample loading buffer because precipitation will result and adversely affect electrophoresis. Do not buffer-exchange directly into phosphate buffer because precipitation will occur.

## Related Thermo Scientific Products

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| **20333** | **Pierce® Protein A Agarose,** 5mL |
| **20398** | **Pierce Protein G Agarose,** 2mL |
| **20421** | **Pierce Protein A/G Agarose,** 3mL |
| **20510** | **Pierce Protein L Agarose,** 2mL |

### Product References


Al-Hallaq, R.A., *et al.* (2002). Association of NR3A with the N-methyl-D-aspartate receptor NR1 and NR2 subunits. *Mol Pharmacol* **62:**1119-27.

Brinkmeyer, S., *et al.* (2004). Reformable intramolecular cross-linking of the N-terminal domain of heparin cofactor II: Effects on enzyme inhibition. *Eur J Biochem* **271:**4275-83.

Deleault, N.R., *et al.* (2005). Protease-resistant Prion Protein Amplification Reconstituted with Partially Purified Substrates and Synthetic Polyanions. *J Biol Chem* **280:**26873-9.

Di-Poï. N., *et al.* (2005). Transcriptional Repression of Peroxisome Proliferator-activated Receptor β/δ in Murine Keratinocytes by CCAAT/Enhancer-binding Proteins. *J Biol Chem* **280:**38700-10.

Nguyen, V.T., *et al.* (2000). Pemphigus vulgaris antibody identifies pemphaxin. *J Biol Chem* **275:**29466-76.

Stokes, R.H., *et al.* (2005). Meningococcal Transferrin-Binding Proteins A and B Show Cooperation in Their Binding Kinetics for Human Transferrin. *Infect Immun* **73:**944-52.




Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.




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