

ImmunoPure[®] Gentle Ag/Ab Buffers

21012	21013	21020	21027	0120W
Number	Description			
21012	ImmunoPure[®] Gentle Binding Buffer , 3.75 liters.			
21020	ImmunoPure[®] Gentle Binding Buffer , 1.0 liter. This buffer is a sodium borate buffer, pH 8.0, containing sodium azide as a preservative.			
21013	ImmunoPure[®] Gentle Elution Buffer , 3.75 liters, pH 6.55.			
21027	ImmunoPure[®] Gentle Elution Buffer , 500 ml, pH 6.55.			

Upon arrival products should be stored at 4° C. Products shipped at ambient temperature.

Products guaranteed for one year from the date of purchase if handled and stored properly.

Introduction

The ImmunoPure[®] Gentle Ag/Ab Buffers ensure optimal binding to protein A followed by safe, near-neutral pH elution (6.55). Phosphate buffers or our ImmunoPure[®] (A) IgG Binding Buffer (21001) are not compatible with the elution buffer and must be avoided. The ImmunoPure[®] Gentle Ag/Ab Binding Buffer should be used with the ImmunoPure[®] Gentle Ag/Ab Elution Buffer for optimal recovery of immunoglobulins from samples, when using protein A chromatography.

The ImmunoPure[®] Gentle Ag/Ab Elution Buffer is designed to be an alternative to low pH elution of proteins from a protein A affinity support. Some monoclonals are believed to be unstable at lower pH conditions. Small conformational changes to the structure of an immunoglobulin may not affect antigen binding or other affinity applications, however they may be affected in other ways, including their susceptibility to enzymatic digestion.

ImmunoPure[®] Gentle Ag/Ab Elution Buffer can be used to dissociate antibody-antigen interactions. The near-neutral pH elution buffer ensures that fragile antibodies or antigens will not be harmed when recovered from affinity supports. The Gentle Elution Buffer eliminates the need to neutralize the pH of samples from the elution.

The Fc-binding fusion protein, protein A/G, also works well with the ImmunoPure[®] Gentle Binding and Elution Buffers.

Note: Avoid the use of phosphate buffers for with this system. The Gentle Elution Buffer will form precipitate in the presence of phosphate. If the sample contains any phosphate, the column must be washed with the 15 column volumes of binding buffer or other non-phosphate buffer prior to addition of the Gentle Elution Buffer. Failure to thoroughly wash out the phosphate can result in precipitate forming on the column, ruining the column and preventing sample recovery.

Instructions for Use

Note: These instructions were developed using the Pierce Protein A. Protein A/G may be substituted for protein A in the protocol.

The immobilized protein A and protein A/G manufactured by Pierce are prepared using a leak-resistant coupling method, ensuring excellent gel stability and binding characteristics toward IgG.

Make Ready

Note: Allow the immobilized protein A and the ImmunoPure® Gentle Ag/Ab Buffers to warm to room temperature before use.

Sample Preparation

1. For serum samples, ascites fluid, or tissue culture supernatant, it is necessary to dilute them at least 1:1 with binding buffer prior to their application to the protein A columns. This is necessary to ensure that the proper ionic strength and pH are maintained for optimal binding.
2. If plasma is used instead of serum, the sample will become hazy after dilution with the ImmunoPure® Gentle Ag/Ab Binding Buffer due to the precipitation of lipoproteins. The diluted plasma sample should be centrifuged at 10,000 x g for 20 minutes and the supernatant applied to the equilibrated protein A column. Good recoveries can be expected from plasma if this procedure is followed.

Standard Protocol for the Isolation and Purification of IgG

1. Equilibrate the immobilized protein A column with 5 column volumes of binding buffer. Do not use a phosphate binding buffer or our ImmunoPure® (A) IgG Purification Binding Buffer. We recommend our ImmunoPure® Gentle Ag/Ab Binding Buffer, which is completely compatible with our ImmunoPure® Gentle Ag/Ab Elution Buffer.
2. Apply the diluted sample to the column and allow it to flow completely into the gel.
3. Wash the protein A column with 15 column volumes of binding buffer.
4. Elute the bound IgG with 5 column volumes of ImmunoPure® Gentle Ag/Ab Elution Buffer. This can be collected as one large fraction or, if a more concentrated sample of IgG is preferred, fractions can be collected. Monitor elution of bound proteins by absorbance at 280 nm.
5. Regenerate the immobilized protein A column by washing with 8 column volumes of 0.1 M citric acid adjusted to pH 3.0 with NaOH. For storage, wash the column with an additional 5 column volumes of water containing 0.02% sodium azide. The ImmunoPure® Immobilized Protein A column may be regenerated a minimum of 10 times without a significant decrease in performance.
6. Perform a buffer exchange by desalting or dialysis to remove excess salt and exchange sample into a more physiological buffer.

Note: Do not buffer exchange directly into a phosphate buffer or a precipitate will form. We recommend borate, TRIS, acetate or carbonate buffers for the initial buffer exchange. Phosphate buffers can be used following desalting with a compatible buffer.