Thermo SCIENTIFIC

Optimize elution conditions for immunoaffinity purification

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Introduction

Affinity purification involves specific noncovalent binding interactions between ligand and target molecules. First, purified ligand molecules are immobilized to a solid support, such as porous agarose beads. Then a complex mixture containing the target molecules (i.e., the molecule to be purified) is added to the solid support, allowing the target molecules to bind by their specific affinity to the immobilized ligand molecules. After washing away nonbound components of the complex mixture, the captured target molecules are released and recovered (i.e., eluted) from the ligand molecules using buffer conditions that disrupt the affinity interaction.

Appropriate buffer conditions for binding and elution steps in affinity purification are as varied as the types of molecules concerned and their chemical binding properties. Unique binding/elution conditions exist for certain affinity systems, such as between lectins and sugars, chelated divalent metals and histidine tags, and substrates and enzymes; however, conditions for binding and elution of antibody-antigen affinity interactions (immunoaffinity) are more predictable. Antibody-antigen binding usually is most efficient in aqueous buffers at physiological pH and ionic strength, such as in phosphate-buffered saline (PBS). Consequently, elution often can be accomplished by raising or lowering the pH or altering the ionic state to disrupt the binding interaction.

Which type of elution condition (pH, ionic strength, chaotrope, or denaturant) is most effective for a particular antibody-antigen interaction depends on the specific composition of ionic, hydrophobic and hydrogen bonds involved. (A chaotrope is a salt that disrupts the structure of water and is often related to ionic strength and/or denaturing effects). The ideal elution buffer effectively releases the antibody or antigen without irreversibly denaturing or inactivating them. In practice, all elution buffers cause some loss of antibody or antigen function, limiting the number of times an affinity support can be reused.

Polyclonal and monoclonal antibodies differ in their interaction with antigens, requiring different strategies for elution. Because any bound protein antigen that remains on an antibody affinity column lowers the column's binding capacity for subsequent rounds of purification, complete elution is desirable. Polyclonal antibodies by definition are a mixture of many antibodies varieties, each of which respond to different epitopes by slightly different mechanisms; therefore, effective removal of the all antigen molecules from a polyclonal antibody affinity column may require several different elution conditions. By contrast, monoclonal antibodies bind one specific epitope by a uniform combination of noncovalent bonds, allowing effective elution by one specific condition.

Elution Buffers for Immunoaffinity Purification

The most widely used elution buffer for affinity purification of proteins is 0.1 M glycine•HCl, pH 2.5-3.0. This buffer effectively dissociates most protein-protein and antibody-antigen binding interactions without permanently affecting protein structure. Nevertheless, some antibodies and proteins are damaged by low pH, so eluted fractions are best neutralized immediately after recovery by addition of $1/10^{th}$ volume of alkaline buffer such as 1 M Tris•HCl, pH 8.5 to minimize the duration of time in the low-pH condition.

A. Thermo Scientific Elution Buffers

We offer two, ready-to-use elution buffers for use in protein affinity purification systems:

- IgG Elution Buffer (Product No. 21004 and 21009) is a stabilized, pH 2.8, amine-containing buffer that is efficient and suitable for most immunoaffinity purification systems. Like the glycine buffer mentioned above, some antibodies may be damaged by the low-pH condition.
- Gentle Ag/Ab Elution Buffer (Product No. 21013 and 21027) is a near-neutral (pH 6.6), high-salt buffer for use when low-pH elution buffers adversely affect antibody and/or antigen function. Depending on the specific affinity interactions involved, this buffer may not be as efficient as the IgG Elution Buffer. In addition, eluted samples require dialysis or desalting into phosphate-free buffers before they can be used in many applications or else precipitation may occur.

B. Alternative Elution Buffers

A variety of immunoaffinity elution buffer options are listed in Table 1. Generally, pH and ionic strength conditions are gentle on antibody and protein function, while denaturants and organics are harsh. Usually, a low-pH condition is the best condition to try first. If low pH elutes efficiently but causes irreversible functional damage, then consider testing an ionic strength buffer. If the low-pH buffer does not elute effectively, then consider testing one of the high-pH conditions. When setting up an affinity column for repeated purification of antigen or antibody (especially if it involves using a monoclonal antibody, see previous discussion), consider testing a variety of elution buffer conditions to find one that is as gentle as possible while still being effective.

Table 1. Summary of elution conditions commonly used for immunoaffinity and protein-protein affinity purification.

Condition	Examples
Low pH	IgG Elution Buffer (Product No. 21004 and 21009)
	100 mM glycine•HCl, pH 2.5-3.0
	100 mM citric acid, pH 3.0
High pH	50-100 mM triethylamine or triethanolamine, pH 11.5
	150 mM ammonium hydroxide, pH 10.5
	0.1 M glycine•NaOH, pH 10.0
Ionic strength (and chaotropic effects)	Gentle Ag/Ab Elution Buffer (Product No. 21013 and 21027)*
	5 M lithium chloride
	3.5 M magnesium or potassium chloride
	3.0 M potassium chloride
	2.5 M sodium or potassium iodide
	0.2-3.0 M sodium thiocyanate
	0.1 M Tris-acetate with 2.0 M NaCl, pH 7.7
Denaturing	2-6 M guanidine•HCl (also counts as chaotropic)
	2-8 M urea (also counts as chaotropic)
	1.0 M ammonium thiocyanate
	1% sodium deoxycholate
	1% SDS
Organic	10% dioxane
	50% ethylene glycol, pH 8-11.5 (also counts as chaotropic)
Competitor	> 0.1 M counter ligand or analog

* Gentle Ag/Ab Elution Buffer will form an insoluble precipitate with phosphate buffers (especially those containing potassium phosphate). If phosphate buffers are avoided, no special binding and wash buffer is required for use with the Gentle Ag/Ab Elution Buffer. Note that the Gentle Ag/Ab Binding Buffer (Product No. 21020 and 21012) is intended as a nonphosphate substitute for the regular Protein A Binding Buffer; the Gentle Ag/Ab Binding Buffer (pH 8.0) is not necessarily optimal for other types of antibody-antigen affinity purification systems.

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