

# Cleaning ProSep<sup>®</sup>-A Protein A Affinity Chromatography Media

*Recommendations for minimizing or eliminating non-specific binding and for cleaning ProSep-A media*

ProSep-A protein A affinity chromatography media provide high throughput purification for monoclonal antibodies combined with long lifetime and broad process flexibility<sup>(1)(2)</sup>. ProSep-A, in common with all chromatography media, can exhibit instances of non-specific binding. Such non-specific binding is characterized as the binding of any non-immunoglobulin species present in the feed. In general, the levels of non-specific binding to ProSep-A are very low and the purity of the eluate can routinely exceed 98%. In some cases, a more precise form of "non-specific binding" has been reported. In these cases, rather than "general" non-specific binding the problem is due to a particular component of the feed that binds and co-elutes with the immunoglobulin. This note provides recommendations on how to minimize or eliminate non-specific binding and makes some general recommendations for cleaning ProSep-A media.

## Mechanism of Non-Specific Binding

In the case of ProSep-A, non-specific binding is generally due to two mechanisms, either ionic interaction with unblocked silanol groups on the controlled pore glass base matrix or to a lesser extent hydrophobic interaction with the immobilized ligand coupling<sup>(3)(4)</sup>.

## Recommendations for Addressing Non-Specific Binding

Millipore has screened a wide range of buffers, salts and other potential agents and assessed their potential for minimizing non-specific binding. A post load wash incorporating NaCl (1.0M) was found to be the most generally effective. Other salts, for instance Na<sub>2</sub>SO<sub>4</sub>, were found to be less efficient, requiring higher concentrations. This in turn could promote undesirable hydrophobic binding.

In the case of non-specific binding due to ionic interactions, adding salt in the post-load wash is therefore proposed. The following buffers are recommended:

**Load** – feed buffer typically 50 – 100 mM PBS (phosphate buffer saline) pH 7.5

**Wash** – 50mM PBS with 1.0M NaCl 2 – 5 CV (column volumes)

**Elute** – typically 50mM acetate buffer pH 3.5 (pH dependent on specific MAb (typically 2 – 3 CV)

**Regenerate** – phosphoric acid pH 1.5 (500 mM) (5 CV)

Re-equilibrate in loading buffer (5 – 10 CV)

In the case of non-specific binding that is not addressed by a high salt wash, and is likely due to hydrophobic interaction, a detergent wash using 0.1% Tween® is recommended, utilizing the following:

**Load** – feed buffer typically

50 – 100mM PBS (phosphate buffer saline) pH 7.5

**Wash** – 50mM PBS with 0.1% Tween detergent (2 – 5 CV)

**Elute** – typically 50mM acetate buffer pH 3.5 (pH dependent on the specific MAb (typically 2 – 3 CV)

**Regenerate** – phosphoric acid pH 1.5 (500mM) (5 CV)

Re-equilibrate in loading buffer (5 – 10 CV)

### Cleaning Recommendations

The use of low pH (pH 1.5) regeneration every cycle has been found to be very effective at removing strongly bound material from ProSep-A media. Both protein A and the controlled pore glass base matrix are stable in prolonged exposure to low pH. In contrast, both protein A and the glass base matrix exhibit limited stability at high pH. Where phosphoric acid

alone is insufficient, and a stronger cleaning regime is required, then a periodic treatment with 2 – 6M guanidine hydrochloride is recommended (5 CV). Frequency of treatment depends on the degree of fouling but typically every 5 – 10 cycles is found to be effective. If discoloration of the media, due to very dirty feeds, is experienced, the use of ethanolic acetic acid has been found to be useful. A stepwise elution wash beginning with 20% ethanol/0.5M acetic acid is recommended. It is possible to use higher concentrations of ethanol if necessary but this may pose issues requiring explosion proof operation if large volumes are needed. Twenty percent ethanol does not require explosion proofing in most facilities.

### Applications Support

Should you have non-specific binding or cleaning issues that are not addressed by the above recommendations, Millipore's Chromatography Media Application Group can assist you. Please contact your local Millipore representative for more details on how to access this service.

### References:

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4. Sulkowski, E. (1987) Controlled pore glass chromatography of proteins: Protein purification micro to macro. *Proceedings of a Cetus-UCLA symposium.* Richard Burgess, New York AR Liss. 68 177 – 195

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