General remarks for Affinity Material carrying immobilized Nucleotides

Binding capacity of the column

It is impossible to predict a precise capacity of the material, because this very much depends on the protein itself and on other compounds contained in the mixture to be purified. The degree of substitution, which allows a very rough estimate of binding capacity, is given in the product data sheets.

Handling of Agarose and Toyopearl AF-650M affinity material

For stability of the nucleotide moiety, the material must be kept at low temperature (4°C) and within a pH range of 6.0 to 8.5 during all operations! Long-term storage at pH 7.5 at 4°C with 20% Ethanol.

Toyopearl AF-650M may be used at higher pressure (p_{max}= 3 bar) compared to Agarose (p_{max}= 0.25 bar).

Regeneration of the affinity material

Since the specific effects of other components or impurities in crude mixtures onto the material can not be predicted we specify the material for single use only. We do not recommend using affinity resins multiple times.

Please note: If your sample contains enzymes (even traces) that are able to degrade nucleotides (such as phosphatases), the material may show less or no affinity for nucleotide binding proteins. For example, ATP can be degraded to ADP by phosphatases and ADP shows no or a largely reduced affinity to many ATP binding proteins. This is an issue particularly with material in which the nucleotide is bound via a different position than the γ-phosphate. However, even γ-linked material can be degraded under certain circumstances.

Nevertheless, if you decide to regenerate the affinity matrix, please read the notes below:

General protocol

- It is recommended to use a buffer with an ionic strength of 0.15 M or greater to prevent unspecific ionic interactions between the solute molecules and the matrix. A flow rate of 15 cm/h is recommended.
- To avoid clogging of column filters, it is recommended to filter or centrifuge the sample before applying it to the column to get rid of precipitated material.
- Before applying the sample, equilibrate the column with at least two column volumes of the sample buffer.
- **Sample application:** It is recommended to use at least a 2fold excess of the Agarose (by degree of substitution, see above) over the protein to be purified.
- **Washing:** Wash the columns with 2 – 3 column volumes of a suitable washing buffer.
- **Elution:** Use 0.1 M solution of the nucleotide in a suitable buffer to elute the nucleotide binding protein from the matrix.

Regeneration (make sure you read the notes above): The material may be attempted for re-use by regenerating with 2–3 column volumes of alternating high pH (0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate + 0.5 M NaCl, pH 6.0) buffers. This procedure should be repeated 3 times followed by re-equilibration.

In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the column with 2-3 column volumes of a non-ionic detergent solution, e.g. 0.1% Triton X-100 followed by at least 2–3 column volumes of distilled water. Wash the column with 2–3 column volumes of storage buffer before storage, or use the same amount of sample

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buffer if you want to start a new purification run.

MPLC columns are delivered with adapters for FPLC Systems of BioRad, GE Healthcare (Äkta) and Pharmacia.

Properties of Agarose material

The following table lists some important properties of Agarose affinity material:

Agarose content	4 %
Bead size	45 - 165 µm
p_{max}	0.25 bar
Degree of substitution	See product data sheets
pH stability (short term)	4 - 9
pH stability (long term)	7.5
Recommended linear fllow rate	11.5 cm/h
Chemical stability	Stable to all solutions commonly used in gel filtration, including 8 M urea and 6 M guanidine hydrochloride. **Not stable in organic solvents!**

Properties of Toyopearl AF-650M material

The following table lists some important properties of Toyopearl AF-650M affinity material:

Particle size distribution (>80 % within range)	40 – 90 µm
Mean pore diameter	1000 Å
p_{max}	3 bar
Degree of substitution	See product data sheets
pH stability (short term)	4 - 9
pH stability (long term)	7.5
Recommended linear fllow rate	60 - 600 cm/h
Resin volume	1 g equals ca. 3.5 ml settled swollen resin volume
Chemical stability	Stable to all solutions commonly used in gel filtration, including 8 M urea and 6 M guanidine hydrochloride. **Not stable in organic solvents!**

Please contact info@jenabioscience.com with questions or inquiries.