

MAbsorbent A2P Column Packing Guidelines

Guidelines for Column Packing MAbsorbent A2P Chromatography Adsorbent

MAbsorbent A2P adsorbent can be packed much like any other chromatography adsorbent and depending on scale and equipment availability, is suitable for slurry and pack in place (PIP) procedures. PIP requires prior knowledge of adsorbent pressure ratings, compression and preferred packing flow rates and is usually determined by the end user. For most scales, slurry packing is recommended and a general procedure for slurry packing is outlined as follows:

1. It is recommended that a homogenous 1:1 distribution of adsorbent and storage solution (20% v/v ethanol) is established by careful mixing of the adsorbent container, by inversion of the container or by use of a paddle stirrer. (Please Note: The use of mechanical stirrers, homogenizers or similar devices is not recommended!). Once a homogenous slurry (1:1) has been achieved, the slurry should be poured down the side of the column into approximately 0.1 column volume (CV) of water or phosphate buffered water (PBW), pH 7.4. Alternatively, the slurry can be pumped into the column using a peristaltic pump.
2. The volume of slurry required depends on the final bed height, the preferred packing flow rate and bed compression. MAbsorbent A2P compresses under flow by 15-20% at 400 cm/hr and this may increase to 20-25% compression at higher packing flow rates.
3. Once the required volume has been added to the column, the top plunger should be lowered to 2-3 cm above the slurry and the flow rate increased to $\geq 1.5x$ operational flow rate. Recommended packing solutions are either water or PBW, pH 7.4. Monitor pressure and bed compression, and when bed compression is complete stop the flow. When buffer flow is stopped, the bed will expand.
4. Importantly, adjust the top plunger to 0.5 cm below the packed bed height observed when the adsorbent was being packed under flow. Avoid the ingress of air.
5. Continue packing at preferred packing flow rate for 2 CV's to confirm no further bed compression.
6. If bed compression does occur repeat (4).

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7. Once packing is complete, adjust flow to operational flow rate and continue to pump through packing solution for 2 CV's. If required, test bed integrity by peak asymmetry analysis.
8. If 0.5M NaOH is being used to clean and sanitize the MAbsorbent A2P during processing, it is recommended that the column is flushed with 2 CV's of 0.5M NaOH. Due to the relatively hydrophobic nature of the A2P ligand itself and the high pKa of the functional groups, contact with NaOH may cause pressure spikes at operational flow rates. It is suggested that MAbsorbent A2P is flushed with 0.5M NaOH at linear flow rates $\leq 0.3x$ operational flow rate. Pressure should be monitored and if high pressure is observed, flow rate should be reduced where possible*.
9. Re-equilibrate the column in suitable buffer until conductivity and pH are back to baseline and processing can begin. Occasionally, shrinkage of the bed can occur when re-equilibrating the adsorbent. This is caused by the reduction in pH from >12 down to neutrality and the subsequent exchange of Na ions on the ligands functional groups with OH ions (and therefore a relative increase in ligand hydrophobicity). If the bed shrinkage is not too large (<1 cm), bio-processing should be unaffected. Confirmation of bed integrity can be confirmed by peak asymmetry analysis.

*If pressure problems persist in 0.5M NaOH, re-slurry the bed and repeat (3). Once the bed height has compressed adjust the top plunger to the **exact** bed height seen before the flow was stopped, **not** 0.5 cm below the packed bed height as before. This should allow the bed more de-compression space when in contact with 0.5M NaOH.

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