CaptureSelect™ Resins: Recommendations for Cleaning

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Overview of cleaning

CaptureSelect™ resin lifetime is dependent on several factors:

- The purification process in which the resin is used
- The type and level of impurities of the load material
- The cleaning protocol used to regenerate the resin

Cleaning refers to the removal of tightly bound impurities that remain bound to the resin after the elution/strip step.

Note: Sanitization refers to the reduction of bioburden from the resin. The sanitization process must be optimized for each application, depending on the specific target and type of organism used in the application.

Because binding can differ between resins, different cleaning protocols may be needed for different resins. The cleaning protocol that is used directly affects the number of cycles for which the resin can be used. A resin that is cleaned with an optimized cleaning protocol can be used for many cycles. A resin that is cleaned with an cleaning protocol that has not been optimized can be limited to <5 uses.

Cleaning and lifetime study considerations

- Perform all cleaning studies with the exact load you will use in the manufacturing purification process. The clarification step (for example, depth filter vs centrifuge) can impact the load.
- Filter the load using a 0.45–0.2 µm filter immediately before loading. Precipitation can occur at 2–8 °C in feed streams.
- Monitor the absorbance at A_{214}, A_{260}, and A_{280} to determine if different impurities are eluting. Absorbance at A_{410} is commonly monitored to analyze turbidity and precipitation of host cell proteins during elution.
- Run all strip/cleaning/regeneration steps in upflow mode.
- During the optimization of cleaning procedures, equilibrate the column with equilibration buffer or water before running each cleaning solution.
- Run a sufficient number of column volumes (CVs) of each cleaning solution (typically 3–5 CVs) to ensure that the column is equilibrated in the cleaning solution. Ensure that the entire cleaning peak is eluted before washing out the cleaning solution with water.
- A static hold can be incorporated in the cleaning protocol to increase the residence time of cleaning solution and minimize the cleaning solution volume.
- Use a low flow rate to wash impurities from the resin.
- If you use more than one solution for cleaning (acidic, chaotropic, caustic solutions) in one procedure, use the caustic solution last to minimize irreversible binding of impurities to the resin.
- Perform a mixing/precipitation study in a glass tube to determine if precipitation of the cleaning solution is occurring on the column. On-column precipitation commonly causes pressure issues and fouling.
- Periodically, perform a blank run (no sample load) with the equilibration, elution, and cleaning steps. If peaks are observed during the elution step, it indicates insufficient cleaning of the resin. Other indicators of insufficient cleaning include changes in pH and conductivity during the blank run, and the observation of peaks at the start of the next cycle.

Cleaning cGMP-labeled resins

CaptureSelect™ resins that can be used in a cGMP environment are listed below. Visit the product page for each resin to obtain cleaning information. You can also obtain Certificates of Analysis (CoAs) from the product page.

Table 1 CaptureSelect™ resins for antibody purification

Resin	Product link
FcXL	https://www.thermofisher.com/order/catalog/product/1943280250#/1943280250
FcXP	https://www.thermofisher.com/order/catalog/product/1943712250#/1943712250
CH1-XL	https://www.thermofisher.com/order/catalog/product/1943462250#/1943462250
KappaXL	https://www.thermofisher.com/order/catalog/product/1943210250#/1943210250
KappaXP	https://www.thermofisher.com/order/catalog/product/1943212250#/1943212250
LambdaXP	https://www.thermofisher.com/order/catalog/product/1943752250#/1943752250

Table 2 CaptureSelect™ resins for protein purification

Resin	Product link
FSH	https://www.thermofisher.com/order/catalog/product/1943180250#/1943180250
hCG	https://www.thermofisher.com/order/catalog/product/1943410250#/1943410250
HGH	https://www.thermofisher.com/order/catalog/product/1943160250#/1943160250
TSH	https://www.thermofisher.com/order/catalog/product/1943562250#/1943562250
tPA	https://www.thermofisher.com/order/catalog/product/1943430250#/1943430250
C-tagXL	https://www.thermofisher.com/order/catalog/product/1943072250#/1943072250
HSA	https://www.thermofisher.com/order/catalog/product/1912970250#/1912970250
FactorX	https://www.thermofisher.com/order/catalog/product/1943702250#/1943702250

Cleaning RUO-labeled resins

CaptureSelect™ resins other than those listed in the previous section are for research use only. Suggested for cleaning solutions for RUO-labeled resins are listed below.

Acidic strip solution	Notes
0.5 M acetic or citric acid	Perform the strip step after the elution step. Include additional cleaning steps as needed. The additional steps may not be needed after every run, but may be helpful after every second, third, or fifth run. **Note:** Citric acid has chelating properties and can remove metal ions that might cause discoloration of the resin.

Cleaning solution	Notes
PAB (120 mM **P**hosphoric acid, 167 mM **A**cetic acid, and 2.2 %(v/v) **B**enzyl alcohol)	PAB is the preferred cleaning solution for agarose-based CaptureSelect™ resins (Rogers *et al*., Journal of Chrom. A, 1216 (2009), pp4589-4596). This acidic buffer has shown effective sanitization for affinity resins that are less stable in caustic solution. Its sanitization properties are comparable with 0.5 M NaOH. PAB is not recommended for POROS™-based CaptureSelect™ resins because the benzyl alcohol in the PAB binds to the POROS™ beads, which affects the UV absorption and causes baseline shifts. Large amounts of ethanol (12 to 15 CV) are needed to replace the PAB bound to the POROS™ resin.
NaOH (≤25 mM)	RUO resins have not been tested with NaOH concentrations >25 mM.
Chaotropic agents: 2.0 M guanidine HCl or 6.0 M urea	—
Isopropanol 20% (v/v)	Isopropanol can be effective in removing discoloration from the resin caused by components in the growth media of the cells (such as Vitamin B12).

Options for minimizing post-run cleaning of RUO-labeled resins

Options for minimizing post-run cleaning include the following:

- Add components to the sample load to minimize discoloration of the resin and the binding of impurities:
  - 10–20 mM EDTA (0.5 M tetrasodium EDTA, pH 8.0) to the sample load to bind metal ions and minimize the discoloration of the resin
  - Glycol, urea, or non-ionic detergents to minimize the binding of impurities
- Remove high levels of impurities with a pre-column step before the capture step. For example, if the load contains a component such as phenol red, including an anion exchange step in flow-through mode can bind phenol red before the feedstream reaches the CaptureSelect™ resin.

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

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Revision	Date	Description
A.0	16 September 2021	New document to provide recommended cleaning procedures for CaptureSelect™ resins.