# AAV9 affinity rapid resin

AFFINITY CHROMATOGRAPHY

The AAV9 affinity rapid resin is designed for capture of capsids from adeno-associated virus serotype 9 (AAV9) from clarified or concentrated, buffer-exchanged harvests. By enabling short residence times and eliminating the need for a tangential flow filtration (TFF) step, the resin supports improved yields and reduced processing time. In contrast to its predecessor, Capto™ AVB resin—which efficiently captures multiple AAV serotypes but lacks binding to AAV9 and requires milder cleaning-in-place (CIP) conditions—this next-generation resin has been engineered specifically for AAV9 purification with enhanced robustness.

The AAV9 affinity rapid resin expands the AAV purification toolbox, offering favorable pressure-flow characteristics, excellent packing performance, and compatibility across a wide range of load levels, allowing flexibility in column sizing. Its short residence time accelerates capture steps without compromising performance. Key features include high dynamic binding capacity (DBC), high product yield, effective impurity removal, and exceptional CIP stability, enabling multiple reuse cycles.

These combined properties make the AAV9 affinity rapid resin a powerful and scalable solution for AAV9 capsid purification, supporting its anticipated role as a leading serotype in future gene therapy applications.

## Key features

High DBC at 1 min residence time, > 5 × $10^{14}$ viral particles (VP)/mL resin.

- Efficient impurity removal (> 3 log) even from clarified feed samples.
- Short residence time of 1 min allows for direct loading of clarified feed.
- Similar performance across a wide range of load levels (5% to 80% of $QB_{10\%}$) allowing flexibility in column sizing.
- High alkali stability with CIP in 0.5 M NaOH enabling > 20 run cycles.
- Supports the use in commercial-scale production, with formats for a wide range of scales up to 2000 L suspension bioreactors and comes with regulatory support file (RSF).



**Fig 1.** AAV9 affinity rapid resin

**Table 1.** Main characteristics of the AAV9 affinity rapid resin

| | |
|---|---|
| Matrix | Rigid, highly cross-linked agarose |
| Ligand | AAV9 affinity ligand (alkaline-stabilized, derived from *E. coli*) |
| Coupling chemistry | Epoxy |
| Particle size, $d_{50V}$ * | ~ 60 µm |
| DBC $QB_{10\%}$ † | > 5 × $10^{14}$ VP/mL resin at 1 min residence time<br>> 1 × $10^{15}$ VP/mL resin at 2 min residence time |
| Operating flow velocity, maximum recommended‡ | 600 cm/h at 10 cm bed height |
| Operating pressure, maximum recommended | 0.3 MPa (3.0 bar) |
| Chemical stability | Stable in aqueous buffers commonly used in affinity chromatography |
| pH stability, operational§ | 2.5 to 12.0 |
| pH stability, CIP¶ | 2.0 to 13.7** |
| Delivery conditions | 20% ethanol |

* Mean particle size of the cumulative volume distribution.
† DBC at 10% breakthrough, determined with pure AAV9-(GFP).
‡ Packed in an AxiChrom™ 300 column with 30 cm i.d. at 10 cm bed height, using buffers with same viscosity as water at 20°C.
§ pH range where resin can be operated without significant change in function.
¶ pH range where resin can be subjected to CIP without significant change in function.
** pH 13.7 corresponds to 0.50 M NaOH.

# Effective purification at short residence time

Affinity capture of AAV9 capsids can be performed directly from a clarified feed (normal flow filtration [NFF]) or optionally from concentrated and buffer exchanged (TFF) feed.

We purified AAV9 from clarified harvested supernatant as described in Table 2. The clarified AAV9 was produced in the ELEVECTA™ transient cell line via triple plasmid transfection in a suspension bioreactor culture. The harvested cells were lysed using detergent (Tween 20, 0.5%), treated with a DNA nuclease, and clarified using a PDK11 depth filter. We froze the clarified feed ($6.8 \times 10^{11}$ VP/mL) at −80°C and after thawing, it was passed through a 0.2 µm Supor™ EAV membrane, before loading onto the AAV9 affinity rapid resin. The dynamic binding capacity at 10% breakthrough ($QB_{10\%}$) of the AAV9 affinity rapid resin at a residence time of 1 min in a 1 mL column was $8 \times 10^{14}$ VP/mL of resin, as measured by frontal analysis using purified AAV9.

**Table 2.** Buffers and run protocol for purification of AAV9 from a clarified feed

| Materials | Description |
|---|---|
| Column with AAV9 affinity rapid resin | 2 mL, 10 cm bed height |
| Chromatography system | ÄKTA pure™ |
| UV detection | 280 nm |
| Equilibration buffer | 20 mM Tris + 200 mM NaCl, pH 7.8 |
| Wash buffer | 20 mM Tris + 200 mM NaCl, pH 7.8 |
| Elution buffer | 100 mM Na-citrate, pH 3.0 |
| CIP solution | 0.5 M NaOH |

| Chromatography run protocol | Buffers and volumes | Flow rate (mL/min) |
|---|---|---|
| Step 1, Equilibration | 5 column volumes (CV) of equilibration buffer. | 2.0 |
| Step 2, Sample loading | 1870 mL of AAV9 clarified feed, $6.8 \times 10^{14}$ VP/mL load (80% of $QB_{10\%}$). | 2.0 |
| Step 3, Wash | 10 CV of wash buffer. | 2.0 |
| Step 4, Elution | 10 CV of elution buffer, collected in 1 mL fractions. | 2.0 |
| Step 5, Equilibration/ neutralization | 5 CV of equilibration buffer. | 2.0 |
| Step 6, CIP | 3 CV of 0.5 M NaOH for 15 min, reversed flow. | 0.4 |
| Step 7, Re-equilibration | 5 CV of equilibration buffer. | 2.0 |

After elution, the eluate pool was neutralized with 1 M Tris pH 9.0 to reach approximately pH 7 and analyzed for AAV9 capsid particle titer (Progen AAV9 ELISA). The eluate pool showed yield of 92% (Table 3) when calculated against total VPs in the loaded starting material.

**Table 3.** Analysis of AAV9 titer in the fractions from the affinity capture run with clarified AAV9 feed (Fig 2)

| Sample | AAV9 titer (VP/mL) | Volume (mL) | Total VPs | Yield (%) |
|---|---|---|---|---|
| Start | $6.8 \times 10^{11}$ | 1870 | $1.3 \times 10^{15}$ | |
| Flow through (FT) | $2.5 \times 10^{8}$ | 1870 | $4.7 \times 10^{11}$ | 0.03 |
| Eluate | $1.76 \times 10^{14}$ | 6 | $1.2 \times 10^{15}$ | **92** |
| CIP | $5.06 \times 10^{11}$ | 6 | $6.8 \times 10^{12}$ | 0.5 |

We assessed purity of the eluate by SDS-PAGE analysis using Cy™5 fluorescent dye prelabeling (Amersham™ QuickStain protein labeling kit, limit of detection [LOD] 50 pg) and the results show high purity of the eluate, with no detected impurity bands (Fig 2).

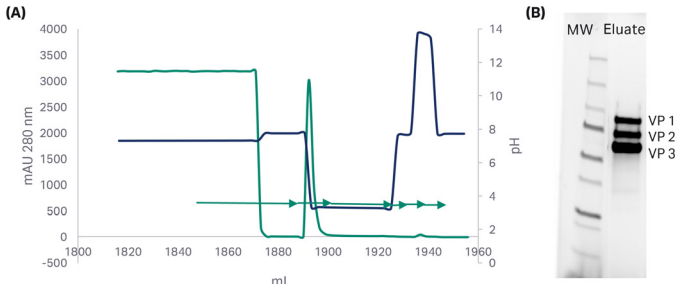


**Fig 2**. Clarified AAV9 feed was applied to a 2 mL column of AAV9 affinity rapid resin and run as described in Table 2. (A) Chromatogram shows last phase of loading, wash, elution, equilibration, CIP, and re-equilibration (indicated by arrows). AAV9 was fully eluted within 2 CV. (B) SDS-PAGE analysis with Cy5 prelabeling confirmed high purity of virus protein bands. MW = molecular weight.

The gel image was captured using Amersham ImageQuant™ 800 Western blot imaging system with sensitive detection and a high contrast setting for visibility of very low level protein impurity bands. We analyzed the eluate for process-related impurities such as host cell proteins (HCPs) and host cell DNA, leached ligand (assays), and AAV9 aggregation by size exclusion, as shown in Table 4.

**Table 4.** Analysis of impurities, leached ligand, and aggregate level in the eluate from the affinity capture run with clarified AAV9 feed (Fig 2)

| Impurity | Method | Concentration |
|---|---|---|
| HCP | HEK293 HCP CL Assay Reagent Kit (Gyros Protein Technologies) | 87 ng/1 × $10^{14}$ VP |
| DNA reduction | Total dsDNA[1] | > 3.2 log reduction |
| Leaked ligand | Enzyme linked immunosorbent assay (ELISA)[2] | 3.8 ng/1 × $10^{14}$ VP |
| Aggregates | Size exclusion chromatography (SEC)[3] | Not detected |

[1] Quantitated with Quant-iT PicoGreen dsDNA Assay kit from Thermo Fisher Scientific.

[2] Quantitated by ELISA using MonoRab VHH antibodies from Genescript.

[3] Identified using analytical SEC with Superose™ 6 Increase 10/300 GL column on Agilent 1260 UHPLC system and fluorescence detection at excitation 280 nm and emission 330 nm.

The same run protocol was also tested on AAV9 sample feed that had been concentrated and buffer exchanged into equilibration buffer by TFF. The chromatogram on Figure 3 shows the loading of 248 mL AAV9 sample (4.6 × $10^{12}$ VP/mL), onto a column packed with 5 mL of AAV9 affinity rapid resin. The eluate yielded 89% AAV viral particles (ELISA), and SDS-PAGE analysis confirmed eluate purity with no visible protein impurities (Fig 3).

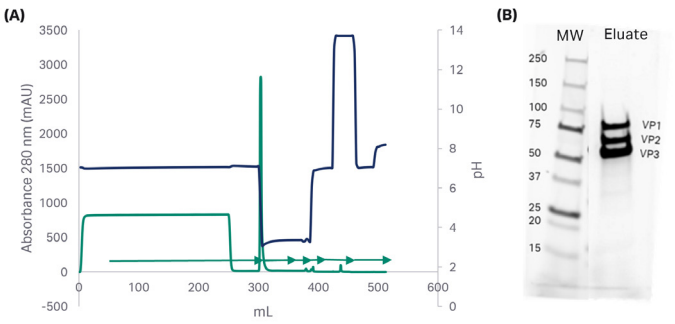


**Fig 3.** Concentrated and buffer exchanged (TFF) AAV9 feed was applied to a 5 mL column of AAV9 affinity rapid resin and run as described in Table 2. (A) Chromatogram shows last phase of loading, wash, elution, equilibration, CIP, and re-equilibration (indicated by arrows). The AAV9 was all eluted within 2 CV. (B) SDS-PAGE analysis with Cy5 prelabeling confirmed no detectable impurities. MW = molecular weight.

## High DBC affected by residence time

Dynamic binding capacity (DBC) at 10% breakthrough ($QB_{10\%}$) was determined using purified AAV9 applied to a 1 mL column packed with AAV9 affinity rapid resin. The column was operated at a range of flow rates corresponding to different residence times, and the resulting $QB_{10\%}$ values are summarized in Table 5. When plotted against residence time, the DBC showed an approximately linear dependence, with lower $QB_{10\%}$ values observed at higher flow rates and shorter residence times (Fig 4).

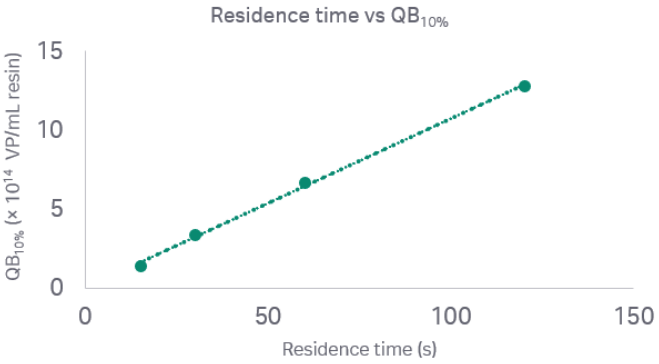


**Fig 4.** DBC at $QB_{10\%}$ for AAV9 affinity rapid resin at different residence times using purified AAV9.

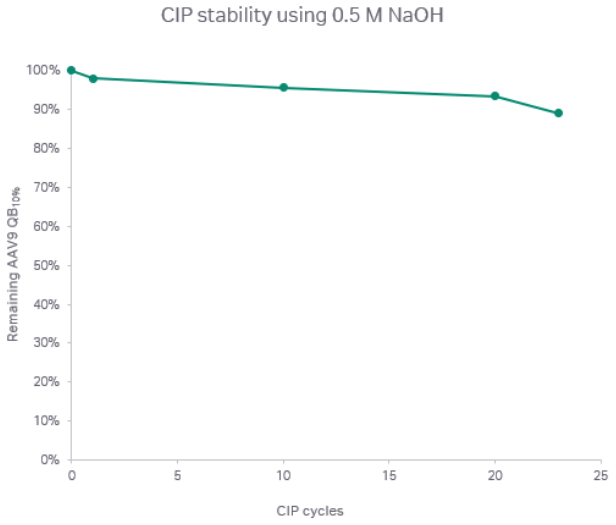


**Fig 5.** Remaining DBC for the AAV9 affinity rapid resin across 23 cycles of AAV9 clarified feed with CIP for 15 min between each cycle.

We observe a high binding capacity at a residence time of 60 s, reaching 6.7 × $10^{14}$ VP/mL resin.

These results demonstrate that the AAV9 affinity rapid resin maintains high binding capacity even at relatively short residence times.

**Table 5.** DBC at $QB_{10\%}$ for AAV9 affinity rapid resin at different residence times using purified AAV9

| Residence time (s) | DBC AAV9, $QB_{10\%}$ (VP/mL resin) |
|---|---|
| 120 | $1.3 \times 10^{15}$ |
| 60 | $6.7 \times 10^{14}$ |
| 30 | $3.4 \times 10^{14}$ |
| 15 | $1.5 \times 10^{14}$ |

## Exceptional alkaline stability for robust resin CIP

Due to its low cost and effectiveness, sodium hydroxide (NaOH) is widely used for cleaning in the bioprocessing industry. Its ability to inactivate endotoxins and a broad range of microorganisms helps minimize the risk of cross-contamination and batch-to-batch carryover. However, repeated exposure to NaOH can gradually reduce DBC, ultimately impacting process productivity. Therefore, maintaining high DBC following NaOH exposure is essential to sustain optimal productivity.

Alkaline stability for the AAV9 affinity rapid resin was evaluated in a cycling study with AAV9 clarified feed. After each run cycle, with CIP using 0.5 M NaOH and 15 min contact time (as in Table 2), DBC was determined as above with purified AAV9 and 1 min residence time and a 0.4 mL Tricorn™ column packed with AAV9 affinity rapid resin (Fig 5). After 23 run cycles, the remaining DBC was approximately 89%, demonstrating exceptional alkaline stability (total contact time in 0.5 M NaOH was 5 h 45 min).

## Reproducible lot-to-lot performance

Three independent lots of AAV9 affinity rapid resin were manufactured and evaluated for AAV9 purification in accordance with the procedure described in Table 2. The chromatogram in Figure 6 presents an overlay of the purification profiles for the eluate peaks obtained from three resin lots, demonstrating performance consistency across resin batches. Fluorescence SDS-PAGE analysis (Cy5 prelabeling) of the three resin lots showed consistent intensity of eluate AAV protein signal and similar purity profiles (Fig 6), as well as VP elution yield by ELISA analysis (Fig 7).

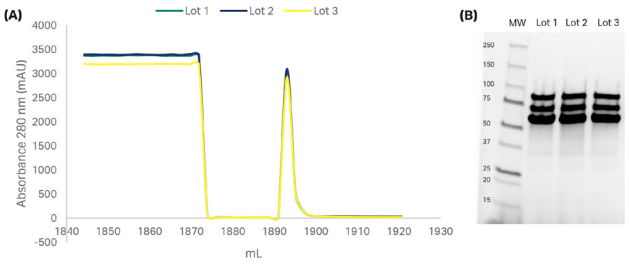


**Fig 6.** (A) Chromatograms show a consistent elution profile from three resin lots of AAV9 affinity rapid resin. The AAV9 was all eluted within 2 CV. (B) SDS-PAGE analysis with Cy5 prelabeling confirmed low levels of detectable protein impurities.

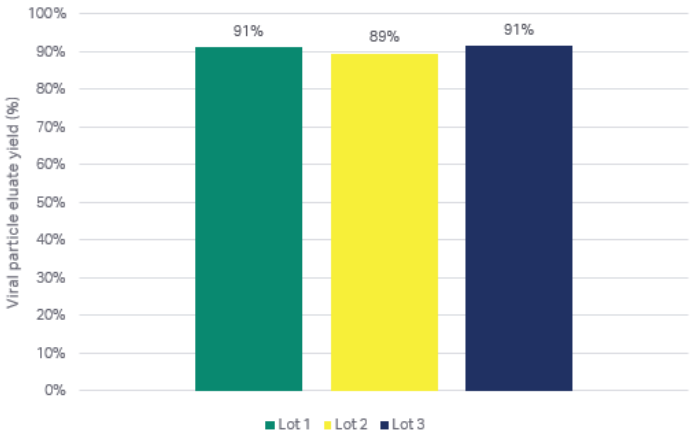


**Fig 7.** Consistent elution yield (%) from three resin lots of AAV9 affinity rapid resin based on VP analysis by ELISA.

## Excellent pressure-flow properties and column packing

The AAV9 affinity rapid resin is compatible with column formats spanning from milliliter to multiliter scales. The pressure-flow characteristics of the resin, when packed in an AxiChrom column with a 30 cm internal diameter, are shown below (Fig 8), determined in water at 20°C (including back-pressure contributions from the column hardware). The recommended bed height for AAV9 purification processes is 10 cm and flow velocity up to 600 cm/h. These parameters ensure optimal performance, supporting efficient processing and scalability across different production volumes.

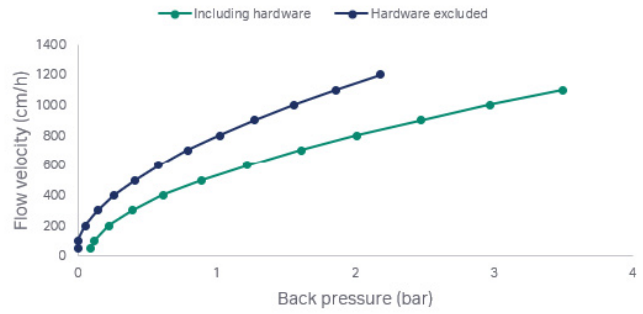


**Fig 8.** Pressure-flow curves for AAV9 affinity rapid resin packed in an AxiChrom 300 column at 10 cm bed height, measured in water at 20°C with and without back-pressure contributions from the column and system hardware. Recommended maximum operating flow velocity is 600 cm/h at 10 cm bed height.

# Seamless scalability

The AAV9 affinity rapid resin is designed for use across bioreactor scales and a wide range of AAV9 titers, supporting both small- and large-scale production. The resin enables direct purification of AAV9 and engineered variants from a clarified feed, eliminating the need for a concentration step and reducing process time through high flow rates and short bed heights. This flexibility supports integration into a variety of manufacturing workflows while maintaining consistent purification performance across different batch volumes and viral particle concentrations.

Table 6 and 7 summarize typical bioreactor sizes for two AAV9 titer ranges and recommend corresponding column dimensions for purification; these can be adjusted to meet specific process requirements. Because performance is maintained across load levels from 5% to 80% of $QB_{10\%}$, the resin also allows flexibility in column sizing, including column oversizing to shorten capture step time.

All Cytiva columns are fully compatible with the ÄKTA™ chromatography system platform.

**Table 6.** Recommended column sizes and supporting ÄKTA instruments for AAV9 titers ~ $1 \times 10^{12}$ VP/mL. Proposed column size is based on a load of $5 \times 10^{14}$ VP/mL resin and full capacity utilization (80% of $QB_{10\%}$), 1 min residence time, and feed load time of max 4 h

| Bioreactor size per cycle (L) | Phase | Proposed column and size | System compatibility |
|---|---|---|---|
| 0.1–0.25 | PD | AAV9 affinity rapid resin column, 1 mL | ÄKTA pure, ÄKTA avant, ÄKTA go™, ÄKTA start™ |
| 0.25–1.0 | PD | AAV9 affinity rapid resin column, 4.7 mL | ÄKTA pure, ÄKTA avant, ÄKTA go, ÄKTA start |
| 1–5 | PD | HiScale™ 16 column (Ø 1.6 cm, 10 cm bed height, 20.1 mL) | ÄKTA pure, ÄKTA avant, ÄKTA go, ÄKTA start |
| 10–50 | PD | HiScale 50 column (Ø 5.0 cm, 10 cm bed height, 196 mL) | ÄKTA pilot™ 600, ÄKTA ready™ 450 |
| 50–200 | PD, GMP | AxiChrom 100 (Ø 10 cm, 10 cm bed height, 785 mL) | ÄKTA pilot 600, ÄKTA ready, ÄKTA process™ |
| 200–750 | GMP | AxiChrom 200 (Ø 20 cm, 10 cm bed height, 3.1 L) | ÄKTA process, ÄKTA ready |
| 750–1700 | GMP | AxiChrom 300 (Ø 30 cm, 10 cm bed height, 7.1 L) | ÄKTA process, ÄKTA ready |

**Table 7.** Recommended column sizes and supporting ÄKTA instruments for AAV9 titers ~ $3 \times 10^{12}$ VP/mL. Proposed column size is based on a load of $5 \times 10^{14}$ VP/mL resin and full capacity utilization (80% of $QB_{10\%}$), 1 min residence time, and feed load time of max 4 h

| Bioreactor size per cycle (L) | Phase | Proposed column and size | System compatibility |
|---|---|---|---|
| 0.1–0.5 | PD | AAV9 affinity rapid resin column, 4.7 mL | ÄKTA pure, ÄKTA avant, ÄKTA go, ÄKTA start |
| 0.5–3.0 | PD | HiScale 16 column (Ø1.6 cm, 10 cm bed height, 20 mL) | ÄKTA pure, ÄKTA avant, ÄKTA go, ÄKTA start |
| 3.0–8.0 | PD | HiScale 26 column (Ø 1.6 cm, 10 cm bed height, 53 mL) | ÄKTA pure |
| 8.0–30 | PD | HiScale 50 column (Ø5.0 cm, 10 cm bed height, 196 mL) | ÄKTA pilot 600, ÄKTA ready 450 |
| 30–130 | PD, GMP | AxiChrom 100 (Ø 10 cm, 10 cm bed height, 785 mL) | ÄKTA pilot 600, ÄKTA ready, ÄKTA process |
| 130–500 | GMP | AxiChrom 200 (Ø 20 cm, 10 cm bed height, 3.1 L) | ÄKTA process, ÄKTA ready |
| 500–1200 | GMP | AxicChrom 300 (Ø 30 cm, 10 cm bed height, 7.1 L) | ÄKTA process, ÄKTA ready |

## How to use the AAV9 affinity rapid resin

The AAV9 affinity rapid resin is available as a bulk resin in units of 25 mL, 100 mL, and 1 L. Compatible with packing in HiScale and AxiChrom columns (see options in Table 6 and 7). The resin will also be available in prepacked 1 mL and 4.7 mL column formats. These formats enable use from lab- to commercial-scale manufacturing.

## Determining ligand leakage

During AAV purification with a protein affinity ligand, ligand leakage can occur. To control the production process and the amount of ligand contaminant in the final drug product, ligand leakage of the AAV9 affinity rapid resin can be measured using immunoassay analytical techniques. AAV9 ligand leakage can be measured with an ELISA assay using Genscript commercial antibodies (capture antibody MonoRab rabbit anti-humanized VHH antibody [product code A02165-200] and detection antibody MonoRab rabbit anticamelid VHH cocktail [HRP, product code A02016-200]). The ligand is available under a nontransferable limited license for analytical purposes for ligand leakage qualification. Contact us for further information.

## Resin storage

The AAV9 affinity rapid resin is delivered in 20% ethanol. Store unused resin in its container between 2°C and 8°C. Ensure that the screw top is fully tightened. Equilibrate packed columns in buffer containing 20% ethanol to prevent microbial growth. After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use.

## Supply-chain stability

We recognize the critical importance of reliable and secure supply to our customers. We are rapidly expanding our robust manufacturing capacity and maintaining open communication with customers to support supply resilience. We recommend that you work closely with your Cytiva representative to forecast demand to support our production planning and manufacturing operations. Find out more about how we support secure supply to the biopharma industry.

## Support and training

The AAV9 affinity rapid resin belongs to the BioProcess™ family of products developed and supported for large-scale manufacture of biopharmaceuticals. This support includes validated manufacturing methods, secure long-term resin supply, and RSFs to assist process validation and submission to regulatory authorities. In addition, Fast Trak™ training and education provide high-level, hands-on training in key aspects of process development and manufacturing. You can also access Cytiva online learning to build your skills and continue your education.

## Ordering information

| Product | Size | Product code |
|---|---|---|
| AAV9 affinity rapid resin | 25 mL | 17542601 |
| AAV9 affinity rapid resin | 100 mL | 17542602 |
| AAV9 affinity rapid resin | 1 L | 17542603 |
| AAV9 affinity rapid resin column, 1 mL | 5 × 1 mL | 17542652 |
| AAV9 affinity rapid resin column, 4.7 mL | 4.7 mL | 17542615 |

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