



# Quick Purification Strategy Adenovirus Purification

Adenoviruses are commonly used as gene transfer vectors in gene therapy and clinical trials. However, their large size, complexity, and acid labile nature lead to challenges in their downstream purification. The following purification strategy provides an easy two-step protocol to purify adenoviruses.

Initial resin screening	UNOsphere™ S (cation exchange)	Nuvia™ S (cation exchange)	Nuvia™ cPrime™ (mixed mode)	UNOsphere™ Q (anion exchange)	Nuvia™ Q (anion exchange)
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## Results

Virus in flowthrough	+++	+++	—	—	—
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Virus in eluate	++	++	++++	++++	++++
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Conclusion	Poorly suited for both capture and polish steps		Considered for direct mass capture		
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Virus binding to Nuvia cPrime requires low salt and pH conditions. Therefore it cannot be used for the polish step after anion exchange, where the resulting feedstream would likely have high ionic strength and high pH.

Nuvia cPrime selected for the mass capture step

Results in ~500 mM NaCl eluate

Nuvia Q can adsorb virus at higher NaCl concentrations relative to UNOsphere Q.

Nuvia Q selected for the polish step

## Results

	Harvest	Nuclease-treated harvest	Nuvia cPrime	Nuvia Q
HCP, ng/10 <sup>10</sup> particles	ND	3,022	58	2
HC dsDNA, ng/10 <sup>10</sup> particles	3,144	30	ND	<0.02
Total virus (x10 <sup>11</sup> particles)	30.6	31.8	18.4	16.4

HCP, host cell protein. HC dsDNA, host cell dsDNA. ND, not determined.

Refer to experimental details on page 2.

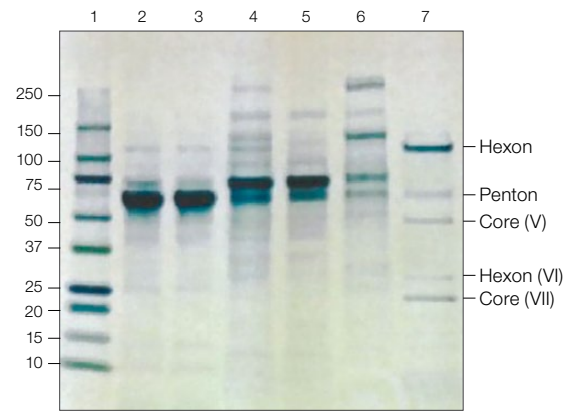
# Adenovirus Purification Strategy Details

## Capture Conditions

Step	Buffer	Column Volume	Flow Rate, cm/hr
Equilibration	25 mM histidine, pH 6.0 (buffer A)	10	120
Sample Loading	Culture supernatant diluted 1:3 with buffer A	48	120
Wash	Buffer A	5	120
Elution	75 mM Tris, 525 mM NaCl, pH 8.5	3	120

## Polish Conditions

Step	Buffer	Column Volume	Flow Rate, cm/hr
Equilibration	75 mM, pH 8.0 (buffer A)	10	120
Sample Loading	Nuvia cPrime eluate diluted 1:1 with buffer A	2	120
Wash 1	75 mM, 250 mM NaCl, pH 8.0	2	120
Wash 2	15 mM Tris, 440 mM NaCl, pH 8.5	3	120
Elution	75 mM Tris, 1M NaCl, pH 7.5	5	120



**SDS-PAGE of intermediates and the final product.** Lane 1, MW marker; lane 2, Nuvia cPrime load; lane 3, Nuvia cPrime flowthrough; lane 4, Nuvia cPrime elution/Nuvia Q load; lane 5, Nuvia Q flowthrough; lane 6, Nuvia Q pre-elution; lane 7, Nuvia Q product.

## Resources

Download [bulletin 6713](#) to get details about this workflow.

Visit [bio-rad.com/web/ProcessResins](http://bio-rad.com/web/ProcessResins) to get technical details about the resins used in this workflow.

Visit [bio-rad.com/web/ProcessApplications](http://bio-rad.com/web/ProcessApplications) to see other applications in which these resins can be used.



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