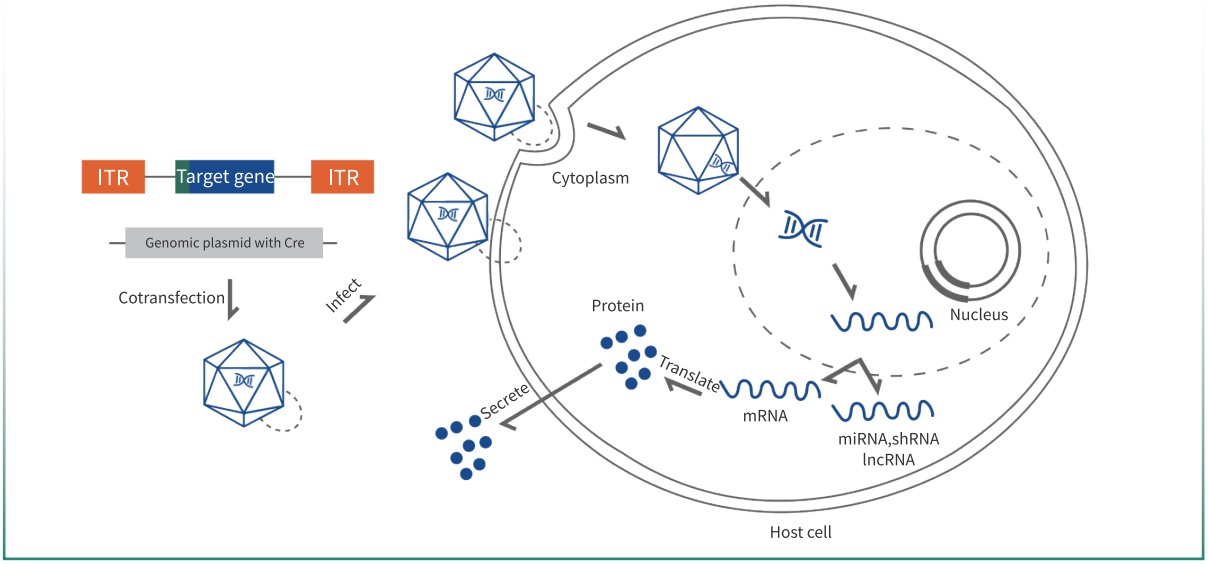
# AAV Purification


## Product Introduction

Adeno-Associated viruses (AAV) belong to the Parvoviridae family and are a group of viruses that cannot replicate autonomously, have a non-pathogenic, non-enveloped icosahedral structure. The virus particles are about 20-30nm in diameter and contain a linear single-stranded DNA genome of about 4.7kb.

AAV is regarded as one of the most promising gene transfer vectors because of its high safety, stable expression of foreign genes, low immunogenicity and wide range of host cells.

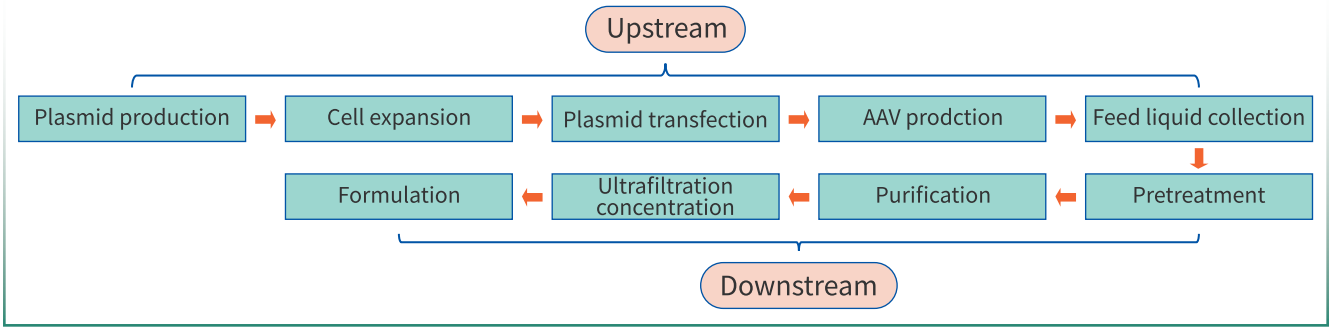
## AAV Infection principle:



# Total process

Nowadays, a number of AAV gene therapy drugs have been marketed, and their industrial production process is a must for major development companies. The upstream process mainly involves plasmid production, cell inoculation and expansion culture, plasmid transformation, virus production and harvest. The challenge lies in scaling up the adhesion and suspension culture systems, as well as in producing high-titer, high-potency viral vectors.

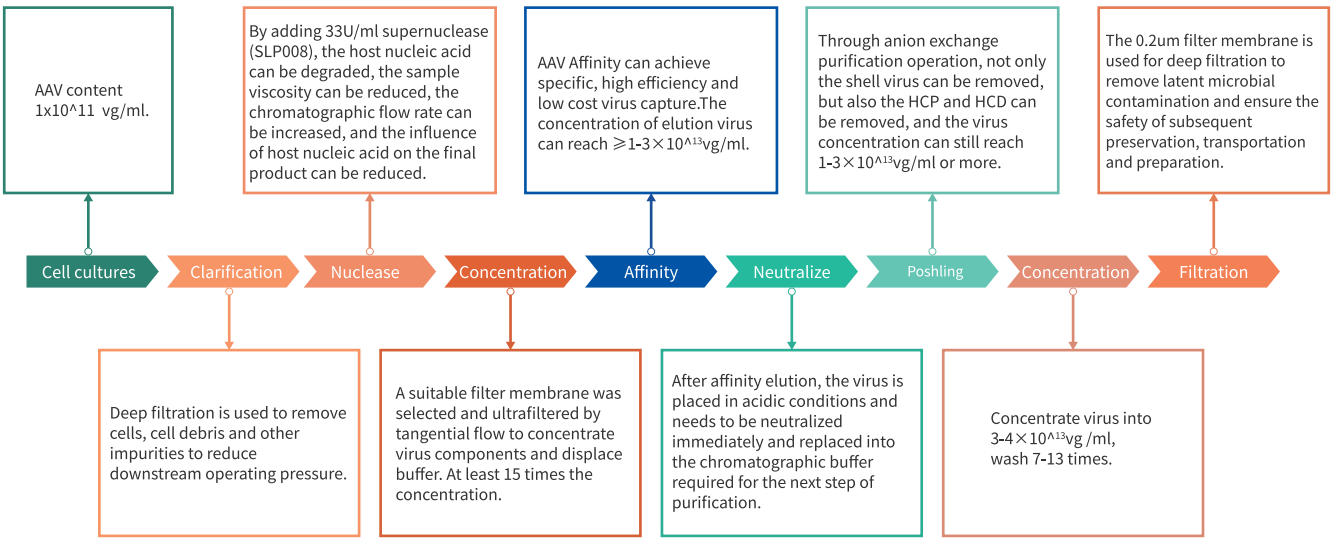
Downstream, a two-step chromatography process is used to purify the virus, and the dead end or tangential flow filtration methods are used to achieve sample clarification, concentration, liquid exchange and sterilization operations, and finally obtain a sufficient amount of virus for inoculation. The challenges lie in the isolation of Empty Capsids and the differences in purification processes of different serotypes.




# AAV downstream process

In various AAV purification processes, ultracentrifugation is one of the earliest and most conventional purification methods, but this method is unable to meet the needs of large-scale industrial production. With the continuous development and improvement of the chromatography process, the current chromatography method has become the first choice for industrial production of AAV.

## AAV production process



## AAV affinity chromatography ( AAV Affinity Beads 4FF) ▶▶▶

AAV Affinity Beads 4FF is used for AAV capture (see Figure 1). From the sample supernatant with titer of 1E10-1E11 vg/ml, high purity virus can be purified with a yield of more than 80%, and the total amount reaches $10^{13}$vg.

AAV Affinity Beads 4FF meet the large-scale purification needs of adeno-associated viruses (AAVS) for gene therapy, binding a range of AAV serotypes, including AAV1 to AAV9 and synthetic serotypes, for excellent purity and yield can be obtained in one step.

### Features:

- ▶ **High versatility:** Suitable for AAV1~AAV9 serotypes
- ▶ **High capacity:** 1E12 ~ 1E14 vg/ml
- ▶ **High yield :** ⩾80%
- ▶ **High tolerance:** Can withstand a certain concentration of acid, lye and organic reagents
- ▶ **High safety:** No animal source components, safe clinical application, in line with regulatory requirements
- ▶ **High stability:** Independent research and development production, quality control from the source, to ensure the stability of product batches
- ▶ **Low cost:** Standard production management, save costs, enhance product competitiveness
- ▶ **Robust amplification:** Can withstand higher flow rates, suitable for large-scale purification

## AAV Anion exchange chromatography (Separate Empty/Full Capsids with Smac Q40) ▶▶▶

The presence of Partial, Empty capsids not only affects the effectiveness of treatment, but may also lead to an immune response, so removing them becomes critical. The affinity chromatography procedure cannot distinguish between intact capsids, empty capsids, incomplete capsids, polymers, etc., and requires fine separation through differences in electricity to remove impurities such as empty capsids as much as possible. Polishing is a resolution challenge, and the small difference in isoelectric point (PI) between intact capsids and empty capsids is utilized. Empty shell separation was achieved by high-resolution anion (Smac Q40) chromatography (see Figure 2).

After affinity chromatography and ion chromatography,impurities such as empty capsid, incomplete capsid and polymers can be effectively removed to obtain intact AAV particles, and the intact capsid rate can reach more than 90%, which meets the demand of AAV drug preparation.

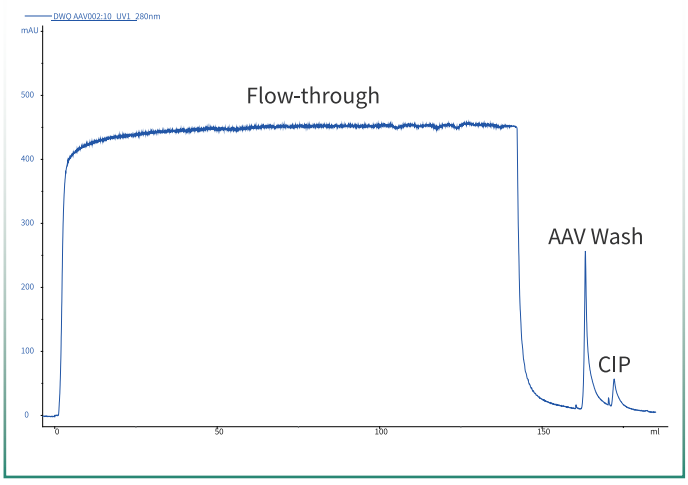



Figure 1. AAV8 chromatographic map by AAV Affinity Beads 4FF

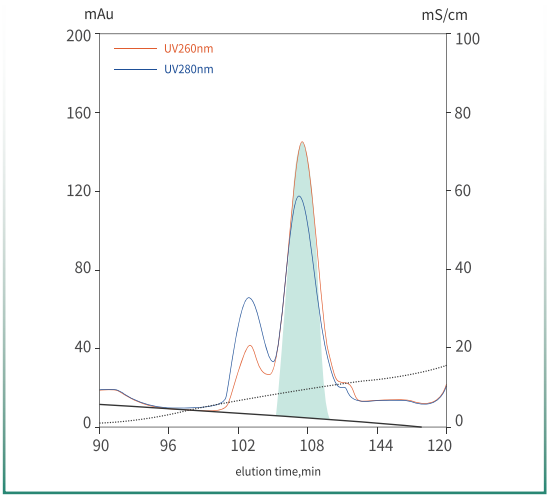



Figure 2. Isolation of AAV8 E/F by Smac Q40

# AAV quality evaluation

The SDS-PAGE (reduction) electrophoretic display of each sample during the affinity chromatography process ( Figure 3), from which the three bands of VP1, VP2 and VP3 of the virus can be clearly seen.

Then, the purified AAV8 virus was diluted in multiple gradients and infected 293T cells (confluent degree > 50%). Since the virus plasmid contained GFP gene, the infected cells could express GFP protein. It was observed by fluorescence microscope that the fluorescence intensity was very high after dilution of 1000 times. With the increase of dilution ratio, the number of AAV infected cells decreased gradually, but there were still a few cells with fluorescence after 10^6 times dilution. The eluted virus proved to be highly active.

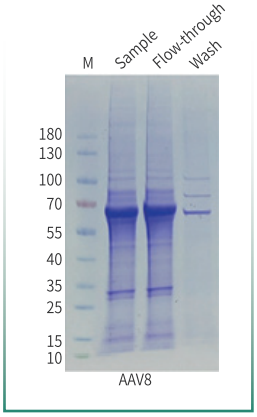



Figure 3 . AAV8 SDS-Page

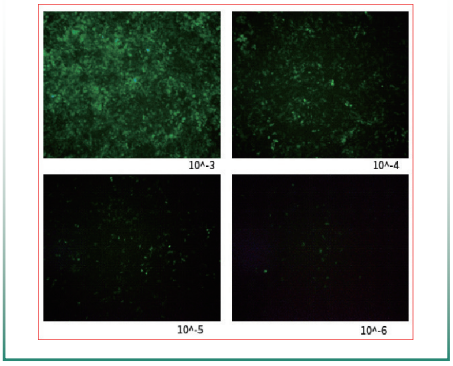



Figure 4. Fluorescence results of 293T cells infected with AAV8 virus eluent diluted $10^3$, $10^4$, $10^5$, $10^6$ times

## Product recommendation

| Phases | Product name | Product code | Application |
|---|---|---|---|
| Capture | AAV Affinity Beads 4FF | SA096 (5ml/25ml/100ml/1L) | AAV affinity beads for capture |
| Polishing | Smac Q 40 | SI035 (25ml/100ml/500ml/1L/10L) | Strong anion exchange, high rigidity agarose matrix, smaller grain diameter, higher column efficiency, suitable for empty solid shell separation |
| | Q 70S/M/L Phmac Beads | SI039/SI056/SI060 (25ml/100ml/500ml/1L/10L) | Polymer matrix strong anion exchange, 100, 500, 1000nm three aperture options, uniform diameter, ultra-high pressure |

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