



Adeno-Associated Virus Production Services

INTRODUCTION

Applied Viromics was established to provide technical expertise and production services of viral vectors for in vitro and in vivo gene delivery. We earned our reputation as reliable provider of viral vectors with our commitment to deliver the best quality viral vectors for your scientific research.

We started to produce recombinant adeno-associated virus (AAV) for researchers since 2001. Now we offer both standard batch production and a larger size custom production. For researchers who would like to test AAV in their labs, we offer free AAV samples expressing GFP and LacZ marker genes.

AAV PRODUCTION OVERVIEW

First, gene of interest needs to be cloned into AAV shuttle plasmid. Applied Viromics provides shuttle plasmids with three different kinds of promoters, together with SV-40 poly A signal. If you would like to use our shuttle plasmids, scientists at Applied Viromics will perform the subcloning and necessary plasmid production for you.

Researchers can also provide their own AAV shuttle plasmids. This way, time will be saved from constructing and preparing plasmids, together with the need to confirm the integrity of plasmids between the researcher and us.

For AAV production, we use the triple-transfection method similar with the one described by Stratagene. Thus for researchers who use Stratagene AAV plasmids, their AAV vector can be produced the same way with our method using those plasmids. So for custom AAV production, we only need our customer to provide us AAV shuttle plasmids. Applied Viromics will use our in-house helper plasmids to do viral production.

Standard batch production is performed by transfecting 20× 150mm dishes, and we guarantee to deliver a minimum 2e12 genome copy (GC) in purified vector. In most of the cases, yield is 2 to 3 -fold higher. For customers who have their own AAV shuttle plasmids, we ask for 1mg of the plasmid to be shipped to us.

Large-scale custom production is designed to produce more than 1e13 GC of purified vector and cell culture scale will be planned based on target yield.

AAV serotype-2 vector is purified by HE and ion exchange column chromatography method developed by Applied Viromics. This method achieves comparable purity of the vector with

double CsCl method and much better recovery. Serotypes other than AAV2 are still purified by CsCl method.

Purified AAV is assayed for titer by measuring viral genome DNA (picogreen assay). Viral vector identity can be checked by PCR method when sequence information is available. Other optional assays are SDS-PAGE/silver staining and endotoxin assay.

Purified AAV is buffer exchanged and sterile filtered. Final concentration of the vector is no less than 1×10^{12} GC/ml. Default formulation of the AAV vector is PBS+0.001% Pluronic F-68.

TIMELINE

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| Gene cloning into Applied Viromics AAV shuttle plasmid Plasmid DNA preparation | 1 week to 10 days |
| Triple transfection and viral production | 1 week to 2 weeks |
| AAV purification and assays | 1 week |
| Document preparation | 2-3 days |

SHIPPING

Final viral products are shipped on dry ice via FedEx priority overnight.

If you are interested in more detail of AAV production service at Applied Viromics, please visit our website at www.appliedviromics.com and call us at (510) 266-2646. We are always there to assist you of your viral vector needs.