Recombinant Adeno-Associated Virus (rAAV) Production with PEI STAR™ Transfection Reagent in Adherent HEK293 Cells

Introduction

Recombinant adeno-associated viruses (rAAVs) are a safe and reliable tool for gene transfer and stable expression in specific human tissues. rAAV production typically begins with three plasmids: one with a transgenic cassette containing the gene of interest and two with AAV packaging genes. These plasmids are co-transfected into HEK293 cells, which will generate infectious rAAVs containing a copy of the transgene. A few days after transfection, rAAVs can be harvested, purified, and used to insert genes into specific tissues.

Polyethylenimine (PEI) transfection is one of the most widely used methods to co-transfect rAAV plasmids into HEK293 cells. PEI STAR™ is more scalable and cost-effective than lipid-mediated transfection, and significantly more reliable than calcium-mediated transfection. PEI STAR™ has been shown to be an effective option for creating highly infectious rAAVs in independent, peer-reviewed literature (Trivedi 2022).

This procedure describes a general method to use PEI STAR™ to prepare rAAVs in a 6 well plate (see Table 1 below for parameters in different vessels). The method can be scaled out to prepare multiple rAAVs and/or scaled up to prepare greater quantities of a desired rAAV. While transfection of an HEK293 suspension is recommended, transfection of adherent HEK293 cells may be faster and more convenient in some situations.

Note: If the AAV genes are on one plasmid instead of two, the plasmid containing the AAV genes should be mixed with the transgene cassette plasmid in equimolar amounts to obtain a pDNA mixture with a concentration of 20 µg / mL, and a final pDNA concentration of 1 µg / mL in the cell culture.

Materials and Equipment

- Equipment for measuring cell concentration, confluence, and viability.
- 6-well plate.
- Incubator set to 37°C, 5% CO_2, 125 rpm, or the most appropriate settings for the cell line.
- 3 x 10^5 HEK293 cells in growth phase.
- 3 mL HEK293 medium.
- 2 sterile 1 mL vials.
- Up to 2.5 µg of transfection-grade plasmids (pDNA) containing transgene cassette and rAAV packaging genes.
- 6.0 µL of PEI STAR™ solution (1 mg/mL) or PEI STAR™ AQ.
- P20, P200, P1000 micropipettes with sterile tips.
- Pipette controller with serological pipette, 10 mL.

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Procedure

1. The day before transfection, trypsinize and count the cells. Plate 3 x 10^5 cells in 2.0 mL of complete growth medium, then incubate. On the day of transfection, cells should be 50% to 80% confluent.
2. Prepare a pDNA mixture in a vial by combining equimolar amounts of all plasmids to be co-transfected, totalling 2.5 µg, diluted in fresh media to 250 µL.
3. Prepare a PEI mixture in a vial by diluting 6 µL of PEI STAR™ solution (1.0 mg / mL) with fresh media to obtain 250 µL of a mixture.
4. Prepare the transfection mixture by combining the pDNA and PEI mixtures and mix by pipetting for a few seconds. Allow the transfection mixture to rest for 5 to 10 minutes before use. *Do not use transfection mixture more than 15 minutes after mixing.*
5. Transfect the cell culture by slowly adding the 500 µL transfection mixture to the well while swirling. Return the cell culture to incubation.
6. rAAVs can be harvested and purified 48 to 96 hours post-transfection, with the appropriate harvest methods depending on the AAV serotype.

Table 1. Table showing parameters for rAAV production in different vessels

Vessel	Plating Medium Volume	Cells per well	Total DNA	Total PEI	Total Dilution Volume
96-well	100 µL	2.5 x 10^4	125 ng	300 ng	20 µL
48-well	200 µL	5 x 10^4	250 ng	600 ng	40 µL
24-well	500 µL	1.25 x 10^5	600 ng	1.5 µg	100 µL
12-well	1.0 mL	2.5 x 10^5	1.2 µg	3.0 µg	200 µL
6-well	2.0 mL	6.25 x 10^5	2.5 µg	6.0 µg	500 µL

References

Huang *et al* (2013) AAV2 production with optimized N/P ratio and PEI-mediated transfection results in low toxicity and high titer for *in vitro* and *in vivo* applications. *J.Virol.Methods* ***193*** 270. PMID: 23791963.

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Trivedi *et al* (2021) Comparison of highly pure rAAV9 vector stocks produced in suspension by PEI transfection or HSV infection reveals striking quantitative and qualitative differences *Mol.Ther.Methods Clin.Dev.* ***24*** 154. PMID: 35071688.

Support

Support is available by emailing us at techsupport@bio-techne.com.