

AAV GFP Production in HEK293F cells - Upstream Process Flow Chart

(using AAV-MAX Helper-Free AAV Production System Kit from Gibco and AAV-DJ Helper Free Expression System from Cell Biolabs)

Innoculate a 125mL Erlenmeyer Flask containing 30mls of complete viral production media with a vial of HEK293F Gibco Viral Production Cells 2.0 (1×10^7 cells)



Monitor cell growth every 24 hours by performing cell concentration and cell viability assays



Subculture the cells when culture density reaches between 4×10^6 - 6×10^6 cells/mL, reduce cell density to 0.3×10^6 - 0.6×10^6 viable cells/ml. Maintain cells in culture for three subcultures (10-14 days) before transfection



Transfect 30mls of cells at a density of 4×10^6 - 6×10^6 cells/mL with a viability of $\geq 95\%$ with AAV2-DJ CMV Expression System (Cell Biolabs plasmids pAAV-CMV-GFP, pAAV-RC, and pHelper) using the TM AAV-MAX Transfection Kit (Gibco)



Harvest cells 70 - 72 hours post-transfection using AAV-MAX Lysis buffer.
Centrifuge at $4000 \times g$ for 30 minutes and collect supernatant (lysate)
Cell Lysate can be stored at 4°C for short duration (i.e. overnight). For long term storage store at -80°C .



Measure AAV titer in cell lysate (optional) and proceed to downstream purification of the AAV particles.