# Adeno-Associated Virus Packaging

## AAV Packaging Protocol

**Table 1: Reagent Requirements for AAV Production**

| Vessel | Seeding Density | DNA Mix | | | Transfection Mix | |
|---|---|---|---|---|---|---|
| | | Transfer Plasmid | Packaging Mix | Serum-Free DMEM | Transfection Reagent | Serum-Free DMEM |
| 15 cm | 12.5 x $10^6$ | 20 µg | 133 µl | 2.5 ml | 80 µl | 2.5 ml |

*Note: the specified amounts and protocol apply to **abm** products and may differ when using alternative products.

### Day 1

1. Seed an appropriate number of HEK293T cells (Cat. No. LV010) into a 15 cm vessel according to Table 1 using complete growth media, and incubate at 37°C with 5% $CO_2$ overnight.

### Day 2

1. Verify that cells have reached 70-80% confluence before proceeding with transfection.
2. Use Table 1 to prepare two solutions, the DNA Mix and Transfection Mix in 1.5 ml tubes. The DNA Mix includes: Transfer Plasmid, Packaging Mix (Cat. No. AAV1001-6) and Serum-Free DMEM (Cat. No. TM003). The Transfection Mix includes: Transfection Reagent (Cat. No. G2500) and Serum-Free DMEM. Incubate at room temperature for 5 min.
3. Prepare the Transfection Complex by combining the DNA Mix and Transfection Mix together, and then incubate at room temperature for 20 min.
4. Add 5 ml of Serum-Free DMEM to the Transfection Complex.
5. Aspirate media from the vessel and gently add the Transfection Complex to the cells. Incubate at 37°C with 5% $CO_2$ for 5-8 h.
6. Add 5 ml of complete growth media to the vessel and incubate at 37°C with 5% $CO_2$ overnight.

**Day 3**

1. Aspirate media from the vessel and add an appropriate amount of complete growth media. Incubate at 37°C with 5% $CO_2$ for 48 h.

**Day 5**

1. Harvest cells and media from the vessel using a cell scraper. Collect in a centrifuge tube and centrifuge at 1500 x g for 15 min at 4°C. Discard the supernatant.
2. Wash pellet by adding 2 ml of sterile PBS to the centrifuge tube and centrifuge at 1500 x g for 5 min at 4°C. Discard the supernatant. Repeat Step 2.
3. Add 2 ml of sterile PBS to the centrifuge tube and gently resuspend cells.
4. Lyse cells by performing three consecutive freeze/thaw cycles using a dry ice ethanol bath and a 37°C water bath.
5. Centrifuge at 10,000 x g for 15 min at 4°C.
6. Apply the clarified supernatant over a PES 0.45 µm sterile filter. Use AAV immediately or aliquot into smaller volumes and store at -80°C.
7. Recommended: Add AAViralEntry™ Transduction Enhancer (Cat. No. G516) to the culture media when using the AAV to enhance downstream transduction efficiency.