Preparation and transfection of AAV-293 Cells

Date:

Investigators:

Plasmid:

Preparation:

Check whether enough plasmid is available: 10 µg of each plasmid per transfection (10 ml cellculture dish).

Switch on the sterile bench.

Is autoclaved water available?

Clean the Ice-box with Ethanol.

Preparation and transfection of cells has to be carried out under **sterile conditions**! Plate the AAV-293 cells at $3 \times 10_6$ cells per 100-mm tissue culture plate in 10 ml of DMEM growth medium 48 hours prior to transfection.

Note: To achieve high titers, it is important that the AAV-293 cells are healthy and plated at optimal density. Cells should be passaged at 50% confluency. It is thus prudent to initially prepare a large number of frozen vials of the cells while they are at a low passage and healthy. Care should be taken to avoid clumping of the cells during passaging and plating for transfection. Cells may be grown to higher confluency prior to transfection with the AAV plasmids.

Transfection:

Note: ***Do not allow the transfection mixture prepared in this section to sit before it is added to the cells.*** *The large aggregates that form after prolonged incubation are inhibitory to uptake.*

1.Inspect the host cells that were split two days before; they should be approximately **70–80% confluent**.

2. Remove the three plasmids to be co-transfected (the recombinant pAAV expression plasmid or control plasmid, pAAV-RC, and pHelper) from storage at –20°C. Adjust the concentration of each plasmid to **1 µg/µl** in sterile/autoclaved millipore water.

Plasmid	**Plasmid concentration in µg/µl**	**Plasmid solution added (µl)**	**End Volume (µl)** min 10 µl
pAAV_____			
pAAV-RC			
pHelper			

3. Pipet **10µg** of each of the three plasmid DNA solutions (**10 µg of each plasmid**) into an eppi. (From now on work at the cellculture bench.)
Fill up to 300 µl with sterile water. Add **300µl** of **0.5 M $CaCl_2$** and mix gently.

4. Pipet **600 µl ml** of **2 × HBS** into a 15-ml falcon.

5. Vortex the falcon (with the 2x HBS) **gently!!!!** while pipetting the DNA/$CaCl_2$ solution dropwise into the falcon (use the Pipetus).

6. Wait 45 min.

7. **Immediately** apply the DNA/$CaCl_2$/HBS suspension to the plate of cells in a dropwise fashion, swirling gently to distribute the DNA suspension evenly in the medium.

8. Return the tissue culture plate to the **37°C room** for **6 hours**.

Note: We strongly suggest performing a viral production negative control by substituting one or all three of the plasmid(s) in the transfection mixture with TE buffer (of the same volume).

7. At the end of the incubation period, remove the medium from the plate and replace it with 10 ml of fresh DMEM growth medium.

8. Return the plate to the 37°C incubator for an additional 66–72 hours.