**Transfection of Viral RNA**

*Walker Orr, 12/12/2023*

## Transfection of RD cells with viral RNA

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| 1. Plate cells and incubate overnight. Cells should be around 80% confluent prior to transfection.    1. 2 million cells in a T25 flask is a good starting point. |
| 1. Prepare reagents:    1. Room temperature OptiMEM.    2. Room temperature TransIT mRNA and mRNA Boost reagent (Mirus, stored at 4C). Vortex gently and spin down before use!    3. 37C DPBS    4. 37C Serum-free DMEM    5. 37C Standard DMEM (10% FBS and Pen/Strep)    6. Thawed RNA, kept on ice. |
| 1. In the cell culture hood, prepare 5 mL 5% FBS DMEM per flask. |
| 1. Change the media in each flask to 5% FBS DMEM. First, wash with DPBS, then add the low FBS DMEM. Return cells to 37C while you prepare the mRNA. |
| 1. Prepare TransIT-mRNA Reagent:mRNA Boost:RNA complexes (immediately before transfection!) as follows (for each flask individually):    1. 630 ul OptiMEM    2. 3.25 ug mRNA    3. 6.5 ul mRNA Boost    4. 6.5 ul Trans-IT   Note that the amounts of RNA, Boost and TransIT are half the amounts recommended by Mirus; we have found that these lower amounts give better viral yields.  After adding each reagent to the tube, pipette up and down with a P1000.  Let stand for 2-5 minutes (3 minutes is good). Then, add dropwise to the corresponding flask. |
| 1. Return flasks to 37C and let stand for 2 days before recovering virus. |