Transfection protocol for HPC7 using TransIT 2020- 96 well plate

Materials:

TransIT 2020, plasmids after we diluted it, OptiMeM reduced-serum medium, growth medium without antibiotics.

A. plate cells

1. Cells ideally should be ≥80% confluent prior to transfection.

Just prior to preparing complexes Plate cells at a density of 2.5‑5.0 × 10^5 cells/ml.

B. prepare TransIT 2020 reagent: DNA complex (Immediately before transfection)

1. Warm *Trans*IT-2020 Reagent to room temperature and vortex gently before using.
2. Place 9 µl (each well) of Opti-MEM I Reduced-Serum Medium in a sterile tube.
3. Add 100ng (2 µl of a 50 ng/µl stock, to each well) plasmid DNA.
4. Pipet gently to mix completely.
5. Add 0.4 µl *Trans*IT-2020 Reagent to the diluted DNA mixture.
6. Pipet gently to mix completely.

7. Incubate at room temperature for 15–30 minutes.

C. Distribute the complexes to cells in complete growth medium

1. Add the *Trans*IT-2020 Reagent: DNA complexes (prepared in Step B) drop-wise to different areas of the wells.

2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the *Trans*IT-2020 Reagent: DNA complexes.

3. Incubate for 24–72 hours. It is not necessary to replace the complete growth medium with fresh medium.

4. Harvest cells and assay as required.