QIAGEN Supplementary Protocol:

Transient transfection of CHO cells in 96-well plates using PolyFect® Transfection Reagent

The following protocol is optimized for transient transfection of CHO cells in 96-well plates.

For more detailed information about PolyFect® Transfection Reagent, and for general guidelines about transfection, please read the *PolyFect Transfection Reagent Handbook* before beginning this procedure.

Important note before starting

- To ensure optimal results we strongly recommend using the optimized amounts of DNA and PolyFect Reagent given in the protocol below.

Procedure

1. **The day before transfection, seed 2 x 10^4 cells per well of a 96-well plate in 100 µl appropriate growth medium.**
2. **Incubate the cells at 37°C and 5% CO_2 in an incubator. The wells should be 40–80% confluent on the day of transfection.**
3. **The day of transfection, dilute 0.5 µg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) with medium containing no serum or antibiotics to a total volume of 30 µl per well. Mix, then spin down the solution for a few seconds to remove drops from the top of the tube.**

   IMPORTANT: Serum and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.
4. **Dilute 2 µl PolyFect Reagent with medium containing no serum or antibiotics to a total volume of 20 µl per well. Add the diluted PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   IMPORTANT: Serum and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.

   Note: It is not necessary to keep PolyFect Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.
5. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation.**
6. **While complex formation takes place, gently aspirate the growth medium from the cells, and wash cells once with 100 µl PBS.**
7. **Add 100 µl cell growth medium (containing serum and antibiotics) to the reaction tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately transfer the total volume to the cells in the 96-well plates.**

   At this stage serum and antibiotics no longer interfere with, but significantly enhance, the transfection efficiency of PolyFect Reagent.

8. **Incubate cells with the transfection complexes for 2–3 h at 37°C and 5% CO_2.**

9. **Remove medium containing the remaining complexes from the cells by gentle aspiration.**

   Note: For optimal results we strongly recommend removing the transfection complexes when working with CHO cells in 96-well plates. Alternatively, transfection-complex removal can be omitted by reducing the amount of PolyFect Reagent to 1 µl and the amount of DNA to 0.25 µg.

10. **Add fresh growth medium (containing serum and antibiotics). Assay cells for expression of the transfected gene after an appropriate incubation time.**

    For example, cells transfected with *β-gal* or *cat* reporter constructs are typically incubated for 24–48 h after transfection to obtain maximal levels of gene expression.