QIAGEN Supplementary Protocol:

Fast-forward protocol for transient transfection of HeLa cells in 96-well plates using PolyFect® Transfection Reagent

The following protocol is optimized for transient transfection of HeLa cells in 96-well plates without pre-plating of cells 24 hours prior to transfection. Cell plating and transfection are performed on the same day, making this protocol rapid and convenient. Two possibilities for transfection-complex formation (in tubes or in the wells of a 96-well plate) are provided in protocol step 2. Please read the protocol thoroughly before beginning this procedure.

Please note that a separate protocol is available for HeLa-S3 cells.

IMPORTANT: Please consult the "General Guidelines" section in 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. **The amounts given are for one well of a 96-well plate.**

Procedure

1. **Dilute 0.4 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 centrifuge for a few seconds to remove any liquid from the top of the tube.**

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

2. **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 Reagent to the DNA solution. Mix, then centrifuge for a few seconds to remove any liquid from the top of the tube.**

   Alternatively, pipet the diluted DNA (step 1) and diluted PolyFect Reagent into one well of a 96-well plate. Mix by pipetting up and down 5 times.

   IMPORTANT: Serum and antibiotics present during this step will interfere with transfection-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.

3. **Incubate the samples for 5–10 min at room temperature (15–25°C) to allow transfection-complex formation. Continue with steps 4 and 5 during this incubation.**

   Note: Transfection-complex formation takes a minimum of 5–10 min. The transfection complexes will remain stable during the time it takes to prepare the cells for transfection; however, avoid extending this incubation for too long.

4. **Harvest the cells by trypsinization and suspend in growth medium (containing serum and antibiotics).**

   Note: The cells should be healthy and in logarithmic growth phase.

5. **Count the harvested cell suspension and adjust the cell density to 3.3–4.0 x 10^5 cells/ml.**

6. **If transfection-complex formation was not performed directly in a 96-well plate (step 2), pipet 50 µl of the solution containing the transfection complexes into one well of a 96-well plate.**

7. **Add 150 µl of the cell suspension (5–6 x 10^4 cells) to wells containing transfection complexes. Mix by pipetting up and down twice.**

   At this stage, the serum and antibiotics present in the growth medium will not interfere with, but rather 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.**

   Note: Cells should have adhered to the plate by the end of this incubation.

9. **Remove medium containing the transfection complexes from the cells by gentle aspiration, and wash cells once with 150 µl PBS.**

   Note: For optimal results, we strongly recommend removing the transfection complexes when using the fast-forward transfection protocol with HeLa cells. If absolutely necessary for a particular application, it may be possible to omit this step by using less DNA and PolyFect Reagent for transfection. As a starting point, we suggest using 0.10 µg DNA and 0.75 µl PolyFect Reagent; however these amounts may need to be optimized to maximize transfection efficiency and minimize cytotoxicity.

10. **Add fresh growth medium (containing serum and antibiotics) and incubate cells at 37°C and 5% CO_2. 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.