



QIAGEN Supplementary Protocol:

Fast-forward protocol for transient transfection of HeLa-S3 cells in 96-well plates using PolyFect® Transfection Reagent

The following protocol is optimized for transient transfection of HeLa-S3 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 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 0.15 μg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 $\mu\text{g}/\mu\text{l}$) 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 $3.3\text{--}4.0 \times 10^5$ cells/ml.**
- 6. If transfection-complex formation was not performed directly in a 96-well plate (step 2), pipet $50 \mu\text{l}$ of the solution containing the transfection complexes into one well of a 96-well plate.**
- 7. Add $150 \mu\text{l}$ of the cell suspension ($5\text{--}6 \times 10^4$ 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 at 37°C and $5\% \text{CO}_2$. 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.

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