QIAGEN Supplementary Protocol:

Transient transfection of HeLa-S3 cells in 60 mm dishes using PolyFect® Transfection Reagent

The following protocol is optimized for transient transfection of HeLa-S3 cells in 60 mm dishes. Parameters for transfection using other culture formats are given in Table 1. **Please note that this protocol is only for HeLa-S3 cells.** Optimized protocols for HeLa cells are provided in the *PolyFect® Transfection Reagent Handbook*.

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 and table below.

Procedure

1. **The day before transfection, seed 8 x 10^5 cells per 60 mm dish in 5 ml appropriate growth medium.**
2. **Incubate the cells at 37°C and 5% CO_2 in an incubator. The dishes should be 40–80% confluent on the day of transfection.**
3. **Dilute 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 150 µl. Mix, then spin down the solution for a few seconds to remove drops from the top of the tube.**

   IMPORTANT: Serum, proteins and antibiotics present during this step will interfere with complex formation and will significantly decrease transfection efficiency.
4. **Add 45 µl PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 s.**

   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 dish, wash cells once with 4 ml PBS, and add 3 ml fresh growth medium (containing serum and antibiotics).**
7. **Add 1 ml 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 60 mm dishes. Gently swirl the dish to ensure uniform distribution of the complexes.**

   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 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.

Table 1. Parameters for transient transfection of HeLa-S3 cells in different formats.

Culture Format	No. of HeLa-S3 cells to seed	Volume of medium‡ (µl)	DNA (µg)	Final volume of diluted DNA (µl)	Volume of PolyFect Reagent (µl)	Volume of medium to add to cells‡ (µl)	Volume of medium to add to complexes‡ (µl)
protocol step	**1**	**1**	**3**	**3**	**4**	**6**	**7**
6-well plate*	4×10^5	3000	2	100	22	1500	600
60 mm dish	8×10^5	5000	4	150	45	3000	1000
100 mm dish†	1.6×10^6	8000	8	300	90	7000	1000

* *When working with 35 mm dishes use the amounts given for 6-well plates*
† *When working with 85 or 90 mm dishes use the amounts given for 100 mm dishes*
‡ *Medium containing serum and antibiotics*

Protocols and handbooks for Transfection Reagents available from QIAGEN can now be downloaded from the Transfection Tools web site —**www.qiagen.com/transfectiontools/**.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.