Quick-Start Protocol April 2016

PolyFect® Transfection Reagent

The PolyFect Transfection Reagent (cat. nos. 301105, 301107, 301108 and 301109) should be stored at 2–8°C upon arrival.

Further information

- *PolyFect Transfection Reagent Handbook*: **www.qiagen.com/HB-2094**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for optimized transfection of COS-7, NIH/3T3, HeLa, 293 and CHO cells in 6-well plates. For transfection using other culture formats, refer to Table 1.
- Cells should be in optimal physiological condition at the time of transfection.
- Plasmid DNA quality strongly influences several transfection parameters, such as efficiency, reproducibility and toxicity. Therefore, plasmid DNA of the highest purity should be used, to ensure consistent transfection results.
- Serum, proteins and antibiotics present during step 3 interfere with complex formation and will significantly decrease transfection efficiency.

Preparation of COS-7, NIH/3T3 and CHO cells

1. The day before transfection, seed 4 x 10^5 cells per well of a 6-well plate with 3 ml appropriate growth medium.
2. Incubate the cells at 37°C and 5% CO_2 in an incubator. The cells should be 40–80% confluent on the day of transfection.

3. Dilute 1.5 µg DNA dissolved in TE buffer, pH 7 to pH 8, (minimum DNA concentration of 0.1 µg/µl) with cell growth medium containing no serum, proteins or antibiotics to a total volume of 100 µl. Mix and centrifuge briefly to remove drops from the top of the tube.
4. Add 10 µl PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times or by vortexing for 10 s. Continue with "Transfection", next page.

Preparation of HeLa cells

1. The day before transfection, seed 4 x 10^5 cells per well of a 6-well plate with 3 ml appropriate growth medium.
2. Incubate the cells at 37°C and 5% CO_2 in an incubator. The cells should be 40–80% confluent on the day of transfection.
3. Dilute 1.5 µg DNA dissolved in TE buffer, pH 7 to pH 8, (minimum DNA concentration of 0.1 µg/µl) with cell growth medium containing no serum, proteins or antibiotics to a total volume of 100 µl. Mix and centrifuge briefly to remove drops from the top of the tube.
4. Add 12 µl PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times or by vortexing for 10 s. Continue with "Transfection", next page.

Preparation of 293 cells

1. The day before transfection, seed 6 x 10^5 cells per well of a 6-well plate with 3 ml appropriate growth medium.
2. Incubate the cells at 37°C and 5% CO_2 in an incubator. The cells should be 40–80% confluent on the day of transfection.
3. Dilute 2 µg DNA dissolved in TE buffer, pH 7 to pH 8, (minimum DNA concentration of 0.1 µg/µl) with cell growth medium containing no serum, proteins or antibiotics to a total volume of 100 µl. Mix and centrifuge briefly to remove drops from the top of the tube.
4. Add 20 µl PolyFect Transfection Reagent to the DNA solution. Mix by pipetting up and down 5 times, or by vortexing for 10 s. Continue with "Transfection", next page.

Transfection

1. Incubate samples for 5–10 min at room temperature (20–25°C) to allow complex formation.
2. While complex formation takes place, gently aspirate the growth medium from each well and add 3 ml fresh cell growth medium (containing serum and antibiotics).
3. Add 600 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 6-well plates. Gently swirl the dish to ensure uniform distribution of the complexes.

   At this stage, serum and antibiotics no longer interfere with transfection and will significantly enhance the transfection efficiency of PolyFect Reagent.
4. Incubate cells with the complexes at 37°C and 5% CO_2 to allow gene expression. Harvest cells and assay for reporter gene expression after an appropriate incubation time.

Table 1. Suggested volumes for transfection using PolyFect Transfection Reagent with various cell lines and multiwell formats

Step	1	1	3	3	4	6	7
Format	Number of seed cells	Medium (ml)	DNA (µg)	Volume diluted DNA (µl)	PolyFect Transfection Reagent (µl)	Medium* added to cells (ml)	Medium* added to complex (ml)
COS-7, NIH/3T3 and CHO cells							
6-well plate	4×10^5	3	1.5	100	10	1.5	0.6
60 mm dish	8×10^5	5	2.5	150	15	3	1
100 mm dish	1.6×10^6	8	4	300	25	7	1
HeLa cells							
6-well plate	4×10^5	3	1.5	100	12	1.5	0.6
60 mm dish	8×10^5	5	3	150	25	3	1
100 mm dish	1.6×10^6	8	6	300	50	7	1
293 cells							
6-well plate	6×10^5	3	2	100	20	1.5	0.6
60 mm dish	1.2×10^6	5	4	150	40	3	1
100 mm dish	2.4×10^6	8	8	300	80	7	1

* Medium containing serum and antibiotics.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.