

**User-developed
protocol**

User-Developed Protocol:

Transient transfection of HEK 293 cells in 75 cm³ flasks using PolyFect[®] Transfection Reagent

This procedure has been developed by customers for the transient transfection of HEK 293 cells in 75 cm³ flasks using QIAGEN[®] PolyFect[®] Transfection Reagent. **It has not been thoroughly tested and optimized by QIAGEN.**

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

Procedure

1. **The day before transfection, seed 2.8×10^6 cells per 75 cm³ flask in 15 ml high-glucose DMEM (containing GLUTAMAX[™] and 4500 mg glucose (Life Technologies, Inc., cat. no. 31966-021); and supplemented with 10% FCS and 1% NEAA (Life Technologies, Inc., cat. no. 11140-035)).**
2. **Incubate the cells at 37°C and 5% CO₂ in an incubator. The flasks should be 60–80% confluent on the day of transfection.**
3. **Dilute 12 µg (range 10–20 µg) DNA, dissolved in distilled water, with growth medium (containing no serum, proteins, or antibiotics) to a total volume of 150 µl, and mix the solution well.**
4. **Add 115 µl PolyFect Transfection Reagent to the DNA solution. Mix by pipetting the total volume up and down at least 5 times.**
5. **Incubate samples for approximately 10 min at room temperature (15–25°C) to enable PolyFect–DNA complexes to form.**
6. **While the complexes are forming, gently aspirate growth medium from the 75 cm³ flask and wash the cell monolayer once with 15 ml PBS.**
7. **Add 7 ml fresh growth medium (supplemented with 10% FCS, 1% NEAA and 2% glutamine) to the flask.**
8. **Add 1 ml growth medium (supplemented with 10% FCS, 1% NEAA and 2% glutamine) to the PolyFect–DNA complexes. Mix well and transfer to the flask. Upon addition, gently swirl the flask to ensure uniform distribution of the PolyFect–DNA complexes.**
9. **Incubate cells with complexes at 37°C and 5% CO₂ for an appropriate time for expression of the transfected gene (typically 24–48 h).**

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.

Trademarks: QIAGEN[®], PolyFect[®] (QIAGEN); GLUTAMAX[™] (Life Technologies, Inc.).
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