



QIAGEN Supplementary Protocol:

Fast-forward protocol for transient transfection of COS-7 cells in 96-well plates using Effectene[®] Transfection Reagent

The following protocol is optimized for transient transfection of COS-7 cells in 96-well plates without pre-plating of cells 24 h 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 3. Please read the protocol thoroughly before beginning this procedure.

IMPORTANT: Please consult the "Safety Information" and "General Guidelines" sections in the *Effectene Transfection Reagent Handbook* before beginning this procedure.

Important note before starting

- To ensure optimal results, we strongly recommend using the optimized amounts of DNA, Enhancer, and Effectene 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 Buffer EC to a total volume of 28.8 μl per well. Add 1.2 μl Enhancer and mix (final volume: 30 μl). Centrifuge for a few seconds to remove any liquid from the top of the tube.**

For example, if the DNA concentration is 0.1 $\mu\text{g}/\mu\text{l}$, dilute 1.5 μl DNA in 27.3 μl Buffer EC, then add 1.2 μl Enhancer.

2. **Incubate at room temperature (15–25°C) for 2–5 min.**
3. **Mix 0.75 μl Effectene Reagent with 19.25 μl Buffer EC. Add the diluted Effectene Reagent to the DNA–Enhancer mixture from step 2. Mix, then centrifuge for a few seconds to remove any liquid from the top of the tube.**
Alternatively, pipet the DNA–Enhancer mixture (step 2) and diluted Effectene Reagent into one well of a 96-well plate. Mix by pipetting up and down 5 times.

Note: It is not necessary to keep Effectene Reagent on ice at all times. 10–15 min at room temperature will not alter its stability.

4. **Incubate the samples for 5–10 min at room temperature to allow transfection-complex formation. Continue with steps 5 and 6 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.
5. **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.

6. **Count the harvested cell suspension and adjust the cell density to $3.3\text{--}4.0 \times 10^5$ cells/ml.**
7. **If transfection-complex formation was not performed directly in a 96-well plate (step 3), pipet 50 μl of the solution containing the transfection complexes into the well of a 96-well plate.**
8. **Add 150 μl 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 significantly enhance, the transfection efficiency of Effectene Reagent.
9. **Incubate cells with the transfection complexes at 37°C and 5% CO₂. 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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