



QIAGEN Supplementary Protocol:

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

The following protocol is for transient transfection of adherent 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 optimization by varying the amounts of DNA, Enhancer, Effectene Reagent, and cells within the ranges given in the protocol below. **The amounts given are for one well of a 96-well plate.**

Procedure

1. **Dilute 0.1 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 29.2 μl per well. Add 0.8 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 1.0 μl DNA in 28.2 μl Buffer EC, then add 0.8 μl Enhancer.

Note: For some cell lines, a higher amount of 0.15 μg DNA provides optimal results. In this case, 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 0.5–1.5 μl Effectene Reagent with 19.5–18.5 μl Buffer EC (final volume: 20 μl). 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.

Note: When working with semiadherent cells, such as 293 cells, we recommend using poly-D-lysine coated 96-well plates.

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.**

Note: For cell types with a small cell size, such as 293 cells, it is necessary to use higher cell densities (e.g., $4.7\text{--}5.3 \times 10^5$ cells/ml).

- 7. If transfection-complex formation was not performed directly in a 96-well plate (step 3), pipet $50 \mu\text{l}$ of the solution containing the transfection complexes into the well of a 96-well plate.**

- 8. 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 significantly enhance, the transfection efficiency of Effectene Reagent.

Note: For cell types with a small cell size, such as 293 cells, it is necessary to use higher cell densities (e.g., $7\text{--}8 \times 10^4$ cells per well).

- 9. 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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