



QIAGEN Supplementary Protocol:

Fast-forward protocol for transient transfection of HeLa-S3 cells in 96-well plates using Effectene[®] Transfection Reagent

The following protocol is optimized for transient transfection of HeLa-S3 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.

Please note that a separate protocol is available for HeLa cells.

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 0.1 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.

2. Incubate at room temperature (15–25°C) for 2–5 min.
3. Mix 1 μl Effectene Reagent with 19 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 $50 \mu\text{l}$ 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.
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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