



## QIAGEN Supplementary Protocol:

### Fast-forward protocol for transient transfection of adherent cells in 96-well plates using Effectene<sup>®</sup> 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  $\mu\text{g}$  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  $\mu\text{l}$  per well. Add 0.8  $\mu\text{l}$  Enhancer and mix (final volume: 30  $\mu\text{l}$ ). 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  $\mu\text{l}$  DNA in 28.2  $\mu\text{l}$  Buffer EC, then add 0.8  $\mu\text{l}$  Enhancer.

**Note:** For some cell lines, a higher amount of 0.15  $\mu\text{g}$  DNA provides optimal results. In this case, if the DNA concentration is 0.1  $\mu\text{g}/\mu\text{l}$ , dilute 1.5  $\mu\text{l}$  DNA in 27.3  $\mu\text{l}$  Buffer EC, then add 1.2  $\mu\text{l}$  Enhancer.

2. **Incubate at room temperature (15–25°C) for 2–5 min.**
3. **Mix 0.5–1.5  $\mu\text{l}$  Effectene Reagent with 19.5–18.5  $\mu\text{l}$  Buffer EC (final volume: 20  $\mu\text{l}$ ). 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  $37^\circ\text{C}$  and  $5\% \text{CO}_2$ . Assay cells for expression of the transfected gene after an appropriate incubation time.**

For example, cells transfected with  $\beta$ -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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