QIAGEN Supplementary Protocol:

Fast-forward protocol for transient transfection of NIH/3T3 cells in 96-well plates using Effectene® Transfection Reagent

The following protocol is optimized for transient transfection of NIH/3T3 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 0.1 µg DNA dissolved in TE buffer pH 7 to pH 8 (minimum DNA concentration: 0.1 µg/µl) 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 µg/µl, 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 1.5 µl Effectene Reagent with 18.5 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 3.3–4.0 x 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 (5–6 x 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% 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.