Quick-Start Protocol April 2016

Effectene® Transfection Reagent

The Effectene Transfection Reagent (cat. nos. 1054250, 301425, 301427 and 1012829) should be stored at 2–8°C upon arrival.

Further information

- *Effectene Transfection Reagent Handbook*: **www.qiagen.com/HB-2084**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for transient or stable transfection of adherent cells in 6-well plates. For transfection using other culture formats, refer to Table 1.
- Cells should be in optimal physiological condition at the time of transfection.
- Plasmid DNA quality strongly influences several transfection parameters, such as efficiency, reproducibility and toxicity. For highest reproducibility and best results with all cell lines, we recommend DNA purified using the EndoFree® Plasmid Kit (cat. nos. 12362 and 12381). This kit quickly and efficiently removes bacterial endotoxins during the plasmid purification procedure, ensuring optimal transfection results.
- The ratio of Effectene Transfection Reagent to DNA required for optimal performance may vary, depending on the cell line and gene target. Table 2 provides suggestions for optimizing the ratio of DNA to Effectene Transfection Reagent. The ratio of DNA to Enhancer (1:8) provided in the protocol should not be changed.

1. The day before transfection, seed 0.9–4 x 10^5 cells (depending on the cell type) per well of a 6-well plate with 1.6 ml appropriate growth medium containing serum and antibiotics.
2. Incubate the cells under their normal growth conditions (generally 37°C and 5% CO_2). The cells should be 40–80% confluent on the day of transfection.
3. On the day of transfection, dilute 0.4 µg DNA dissolved in TE buffer, pH 7 to pH 8, (minimum DNA concentration of 0.1 µg/µl) with Buffer EC, to a total volume of 100 µl. Add 3.2 µl Enhancer and mix by vortexing for 1 s.

   IMPORTANT: Always keep the ratio of DNA to Enhancer (1:8) constant.
4. Incubate at room temperature (15–25°C) for 2–5 min, and then centrifuge briefly to remove drops from the top of the tube.
5. Add 10 µl Effectene Transfection Reagent to the DNA-Enhancer mixture. Mix by pipetting up and down 5 times, or by vortexing for 10 s.

   Note: It is not necessary to keep Effectene Transfection Reagent on ice at all times: 10–15 min at room temperature will not alter its stability.
6. Incubate the samples for 5–10 min at room temperature to allow transfection complex formation.
7. While complex formation takes place, gently aspirate the growth medium from the plate, and wash cells once with 3 ml PBS. Add 1.6 ml fresh growth medium (can contain serum and antibiotics) to the cells.
8. Add 600 µl growth medium (can contain serum and antibiotics) to the tube containing the transfection complexes. Mix by pipetting up and down twice, and immediately add the transfection complexes drop-wise onto the cells in the 6-well plates. Gently swirl the dish to ensure uniform distribution of the transfection complexes.

9. Incubate the cells with the transfection complexes under their normal growth conditions for an appropriate time for expression of the transfected gene. The incubation time is determined by the assay and gene used.

   Optional: In many cases, removal of transfection complexes is not necessary. However, if cytotoxicity is observed, remove the Effectene–DNA complexes after 6–18 h, wash the cells once with PBS and add 3 ml fresh growth medium.

10. **For transient transfections**: Assay cells for expression of the transfected gene. Cells transfected with β-gal or cat reporter constructs are typically incubated for 24–48 h post-transfection to obtain maximal levels of gene expression.

    For stable transfections: Passage cells 1:5 to 1:10 into the appropriate selective medium 24–48 h after transfection. Maintain cells in selective medium until colonies appear.

    Note: We recommend establishing a kill curve (dose–response curve) with each combination of cell line and antibiotic used. It is important to bear in mind that the kill curve can be influenced by cell density. It may be necessary to plate the transfected cells into their normal growth medium (i.e., with no selective drug) and then incubate them for 1–2 days before addition of selective medium.

Table 1. Suggested volumes for transfection using Effectene Transfection Reagent with various multiwell formats

Step	1	3	3	3	5	8
Format	**Number of seed cells**	**DNA (µg)**	**Enhancer (µl)**	**DNA–Enhancer mixture (µl)**	**Effectene Transfection Reagent (µl)**	**Medium added to complex (µl)**
96-well plate	0.5–2 x 10^4	0.1	0.8	30	2.5*	0
48-well plate	1–4 x 10^4	0.15	1.2	50	4*	200
24-well plate	2–8 x 10^4	0.2	1.6	60	5	350
12-well plate	0.4–2 x 10^5	0.3	2.4	75	6	400
6-well plate	0.9–4 x 10^5	0.4	3.2	100	10	600
60 mm dish	2–8 x 10^5	1.0	8.0	150	25	1000

* If transfections are performed in 96- or 48-well plates, dilute Effectene Transfection Reagent with Buffer EC to a total volume of 20 µl or 50 µl, respectively, before addition to the diluted DNA–Enhancer mixture prepared in step 3.

Table 2. Starting points for optimizing transfection of adherent cell lines in 6 well plates

Amount of DNA	0.2 µg	0.2 µg	0.2 µg
Volume of Effectene Reagent	2 µl	5 µl	10 µl
Volume of Enhancer	1.6 µl	1.6 µl	1.6 µl
Amount of DNA	0.4 µg	0.4 µg	0.4 µg
Volume of Effectene Reagent	4 µl	10 µl	20 µl
Volume of Enhancer	3.2 µl	3.2 µl	3.2 µl
Amount of DNA	0.8 µg	0.8 µg	0.8 µg
Volume of Effectene Reagent	8 µl	20 µl	40 µl
Volume of Enhancer	6.4 µl	6.4 µl	6.4 µl

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