Quick-Start Protocol March 2016

TransMessenger® Transfection Reagent

The TransMessenger Transfection Reagent (cat. no. 301525) can be stored at 2–8°C. Stored this way, it is stable for up to 1 year if not otherwise stated on label.

Further information

- *TransMessenger Transfection Reagent Handbook*: **www.qiagen.com/HB-1301**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for transfection of adherent cells using single-stranded RNA in one well of a 6-well plate. For use of other formats, transfection of siRNA and guidelines for optimization, refer to the handbook.
- The cells should be in optimal physiological condition on the day of transfection. Subculture the cells a minimum of 24 hours before transfection. The optimal confluency for transfection is 80–90%.
- Check the integrity and functionality of the RNA.

1. The day before transfection, seed 4–6 x 10^5 cells (depending on the cell type) per well of a 6-well plate in 2 ml appropriate growth medium containing serum and antibiotics.
2. Incubate cells under their normal growth conditions (generally 37°C and 5% CO_2).
3. On the day of transfection, dilute 4 µl Enhancer R in Buffer EC. Add 2 µg RNA (minimum RNA concentration 0.1 µg/µl) and mix by vortexing for 10 s. The final volume should be 100 µl.

   IMPORTANT: Always mix Enhancer R with Buffer EC before adding RNA.

   IMPORTANT: Always keep the ratio of RNA to Enhancer R constant.

4. Incubate at room temperature (15–25°C) for 5 min, and then spin down the mixture for a few seconds to collect drops from the top of the tube.
5. Add 8 µl TransMessenger Transfection Reagent to the RNA–Enhancer R mixture. Mix by pipetting up and down 5 times, or by vortexing for 10 s.
6. Incubate the samples for 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 carefully wash cells once with sterile PBS using 1.5–2 times the volume of medium used for cell seeding.

   IMPORTANT: Do not allow the cells to become dry. Minimize the time they are without medium.
8. Add 900 µl cell growth medium without serum or antibiotics to the tube containing the transfection complexes. Mix by pipetting up and down twice, and then immediately add the transfection complexes drop-wise onto the cells. Gently swirl the plate to ensure uniform distribution of the transfection complexes.

   IMPORTANT: Use medium without serum or antibiotics to avoid RNase contamination.
9. Incubate cells with the transfection complexes for 3 h under their normal growth conditions.
10. Remove the complexes from the cells, wash cells once with PBS and then add 2 ml fresh medium containing serum and antibiotics to the cells.
11. Incubate cells under their normal growth conditions to allow protein expression. The incubation time is determined by the assay and RNA used.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.