siRNA Transfection of Adherent Cells in 384-Well Plates with HiPerFect® HTS Reagent

HiPerFect HTS Reagent (cat. nos. 301802, 301806, and 301807) should be stored at 2–8°C.

For more information, please refer to the *HiPerFect HTS Reagent Handbook*, which can be found at: www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- Cells should be in optimal physiological condition.
- If siRNA has been delivered lyophilized, it must be resuspended prior to transfection.

1. Spot 3.125 ng siRNA in 1–3 µl siRNA Suspension Buffer/RNase-free water into a single well of a 384-well plate (this will give a final siRNA concentration of 5 nM after addition of cells to complexes in step 4).
2. Add 0.1 µl HiPerFect HTS Reagent to 9.9 µl culture medium without serum. Add the diluted HiPerFect HTS Reagent to the prespotted siRNA.

   IMPORTANT: The amount of HiPerFect HTS Transfection Reagent and siRNA required for optimal performance may vary, depending on the cell line and gene target. For suggested starting points for optimization of siRNA to HiPerFect HTS Reagent ratio, see Table 1.
3. Incubate for 5–10 min at room temperature (15–25°C) to allow formation of transfection complexes.

4. Seed 1000–3000 cells in 40 µl of an appropriate culture medium (containing serum and antibiotics) into the well, on top of the siRNA– HiPerFect HTS Reagent transfection complexes.
5. Incubate the cells with the transfection complexes under their normal growth conditions and monitor gene silencing after an appropriate time (e.g., 6–72 h after transfection, depending on experimental setup). Change the medium as required.

Table 1. Starting points for optimizing transfection of adherent cell lines in 384-well plates

Cell line	siRNA concentration (nM/well)	HiPerFect HTS Reagent (µl/well)
A549	5	0.25
AGS	10	0.25
HeLa	25	0.05
HepG2	5	0.1
Huh-7	25	0.25
MCF-7	5	0.1
NIH-3T3	10	0.1
293	10	0.25