

HiFect™: Reverse Transfection Protocol

The following procedure is for transfection of eukaryotic cells in one well of a 96-well plate. For other formats see Table 1. All amounts and volumes are given on a per well basis.

Table 1 lists amounts of HiFect™ used for making an 5:1 ratio (HiFect™ (µl):DNA(µg)). We recommend trying 3:1 and 7:1 ratios additionally. For further optimization other ratios can be tried by using the following equation: **DNA (µg) x Desired Ratio = HiFect™ amount (µl)**.

1. Harvest cells (20.000 – 40.000 per well) and resuspend them in 50 µl fresh culture medium containing twice the normal amount of serum.
Note: Small cells may require higher cell densities (e.g. HEK293 (ATCC® CRL-1573™): 40.000 – 80.000 cells per well).
- 2a. Prepare DNA solution: Add 150 ng of DNA and 25 µl medium for complex formation per well of a 96-well plate (plate A) and mix thoroughly using a pipette.
- 2b. Prepare HiFect™ solution: Mix HiFect™ gently using a pipette before use. Do not vortex. Add 0,75 µl HiFect™ and 25 µl medium for complex formation per well of a 96-well plate (plate B) and mix thoroughly using a pipette.
Important: The medium for complex formation must be serum and antibiotic-free. We recommend using culture medium without serum or Opti-MEM® I Reduced Serum Medium [Invitrogen; Cat. No. 51985].
3. Add DNA solution (plate A) to the HiFect™ solution (plate B) and mix gently by pipetting.
Important: Do not add HiFect™ solution (plate B) to DNA solution (plate A).
4. Incubate HiFect™/DNA complex for 5 minutes at room temperature (20 - 25°C).
5. Add 50 µl of cell suspension to complexes in plate B. Mix DNA complex and cell suspension by gently pipetting up and down twice.
6. Incubate cells at 37°C and 5% CO₂. Incubate cells for at least 18 – 24 hours before analysis.

Remark: Alternatively preparation of DNA solution and HiFect™ solution can be performed in reaction tubes. Therefore add DNA solution (tube A) to HiFect™ solution (tube B) and incubate complexes for 5 minutes. Transfer complexes to culture plate and add cells subsequently.

Remark: It is also possible to plate cells first and add complexes immediately after plating.

Table 1

Culture format	Number of cells per well (reverse transfection)	Number of cells per well (conventional or forward transfection)	Volume of culture medium	DNA solution per well		HiFect™ solution per well		Final HiFect™/DNA complex per well A+B (μl)
				DNA (μg)	Final volume of DNA solution diluted with medium for complex formation A (μl)	HiFect™ (μl) (5:1 ratio)	Final volume of HiFect™ diluted with medium for complex formation B (μl)	
384-well (1 well)	4-8x10 ³	2-4x10 ³	20 μl	0.03	5	0.15	5	10
96-well (1 well)	2-4x10 ⁴	1-2x10 ⁴	100 μl	0.15	25	0.75	25	50
12-well (1 well)	1-3x10 ⁵	0.5-1.5x10 ⁵	1 ml	1	250	5	250	500

Remark: Small cells may require higher cell densities (e.g. HEK293 (ATCC® CRL-1573™): 40.000 – 80.000 cells per well).

Restrictions

HiFect™ Transfection Reagent is sold for laboratory and research use only and shall **under no circumstances** be used for testing or treatment in humans. HiFect™ Transfection Reagent is not a diagnostic or therapeutic product and shall not be used as accessories or complements to such products. The use of the HiFect™ Transfection Reagent is limited for the transfer of nucleic acid only.

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