Manual *IsiFect® in vitro Transfection Reagent*

For research purpose only. Not for therapeutic applications.

Background

IsiFect® is a transfection reagent based on branched Polyethylenimine developed for efficient delivery of nucleic acids into eukaryotic cells *in vitro*. IsiFect® provides highest transfection efficiency of any modified or conventional siRNA as well as DNA in many cell types. IsiFect® shows excellent delivery performance for a wide range of cell types combined with low cytotoxicity. Direct addition of IsiFect®-nucleotide complexes to cells in cultured medium - in the presence or absence of serum - allows fast and easy handling.

Standard Transfection Protocol using IsiFect® Transfection Reagent

The proposed protocol was optimized and calculated for adherent cells in a 6-well plate format. Further optimization and calculation may be required using different cell types and plate formats.

- Step 1: Split cells into 6-well plate 24 hours prior transfection. For transfection cells should display 60-80% confluence.
- Step 2: 1 hour prior transfection remove culture medium and add 1,25 ml fresh medium to the cells.
- Step 3: Dilute 1,5-15 µl IsiFect® in 250µl serum free medium and incubate 5 minutes at room temperature.
  Note: The volume of IsiFect® depends on used cell line. It is recommended to start with a volume of 2,5 µl IsiFect®. Further optimization may be required.
  Add 0,25-50µl siRNA (20µM) to IsiFect®/medium mix and incubate 15 minutes at room temperature.
  Note: The optimal amount of siRNA should be determined empirically. A final concentration of 10–150 nM is recommended.
- Step 4: Add the IsiFect®/siRNA complexes to the cells and mix gently. Incubate for 4 hours.
- Step 5: Add 0,5 ml of medium to each well.

- optional Step: Change medium 24 hours after transfection.

Optimal incubation time for sufficient gene knockdown varies between genes and cell types. Thus, time point of cell harvest should be determined individually.

Besides siRNA transfection, IsiFect® is able to perform DNA transfection and co-transfection of DNA/siRNA into eukaryotic cells. In general, for DNA transfection follow the same protocol as for siRNA and optimize by detecting the respective reporter gene. The recommended amount is 5-20ng DNA/µl IsiFect®.

Cell line index

IsiFect® already was used in a variety of cell lines such as MCF-7, HEK293, Neuro2a, L929, HeLa, HT1080, ME180, SW756, MRI-H-186 and MDA-MB-435.

Safety and storage instructions

For *in vitro* and research purpose only. Not for therapeutic or *in vivo* applications. Store at –20°C for long-term storage; keep working solution at 4°C. Expiry date indicated on the tube.

Optimized Protocol using IsiFect® Transfection Reagent

The proposed protocol was optimized and calculated for MCF7 cells in a 6-well plate format.

- Step 1: Split cells into 6-well plate 24 hours prior transfection. For transfection cells should display 60-80% confluence.
- Step 2: 1 hour prior transfection remove culture medium and add 1,25 ml fresh medium to the cells.
- Step 3: Dilute 6 µl IsiFect® in 236,5 µl serum free medium and incubate 5 minutes at room temperature. Add 7,5 µl siRNA (20µM) to IsiFect®/medium mix and incubate 15 minutes at room temperature.
- Step 4: Add the IsiFect®/siRNA complexes to the cells and mix gently. Incubate for 4 hours.
- Step 5: Add 0,5 ml of medium to each well.

Optimal incubation time for sufficient gene knockdown varies between genes and cell types. Thus, time point of cell harvest should be determined individually.

Troubleshooting

- You cannot detect siRNA delivery? For optimization we advice to use fluorochrome labeled siRNA to detect intracellular delivery and trafficking. Please try several amounts of IsiFect® and siRNA/DNA in order to find the best N/P-ratio. Note that using too much IsiFect® does not inccrease siRNA delivery.
- There is no efficient gene silencing after delivery of siRNA? Most of the used siRNA probably remains probably in the endosome. We advice to optimize the N/P ratio.
- There is no efficient gene silencing by using an optimized protocol: We advise to check siRNA quality and sequence. Additionally please check expiry date.