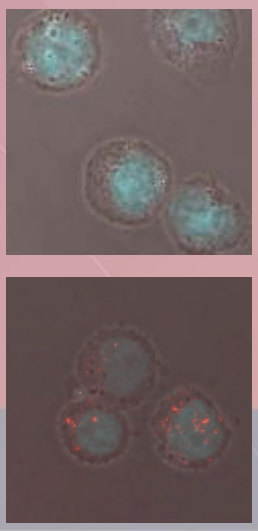
# Tailored Transfection Reagent: *Leu-Fect B*

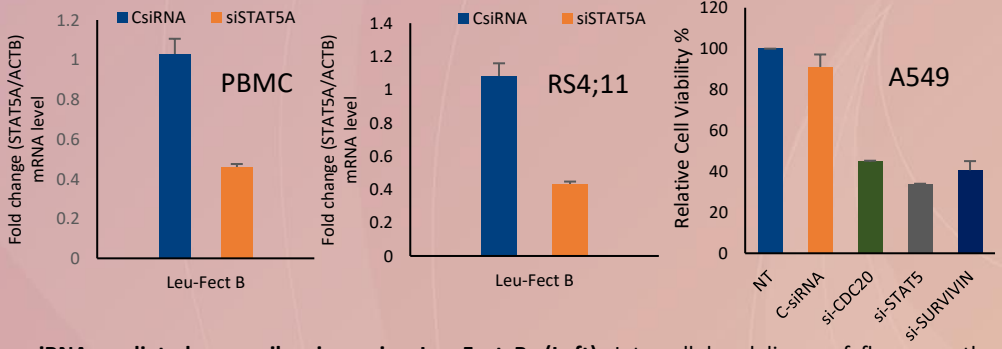
| PRODUCT NUMBERS: 40-10 and 40-20 | SIZE: 0.75 and 1.5 mL | CONCENTRATION: 1 mg/mL | STORAGE: -20 °C |
|---|---|---|---|

## Product Description

**Leu-Fect B** is a highly effective transfection reagent for suspension-growing cells. **Leu-Fect B** is a synthetic amphiphilic polymer that is specifically tailored for siRNA and microRNA delivery. It is capable of undergoing multivalent interactions with nucleic acids and encapsulating co-incubated nucleic acids into ~100 nm particles with a net positive charge. The complexation between the nucleic acids and **Leu-Fect B** occurs in aqueous buffers, obviating the need for organic solvents during preparation. **Leu-Fect B** is a non-integrating carrier of siRNA, so that the genetic make-up of host cells is not altered after treatment with the transfection reagent. **Leu-Fect B** has been tested and found effective in different types of attachment-independent cells, but users are advised to test the efficacy of the reagent in their particular cell type in order to choose the right formulation for long-term use. As with all transfection reagents, formulations of **Leu-Fect B** may need to be optimized for specific cell types/nucleic acids.








**siRNA-mediated gene silencing using Leu-Fect B. (Left):** Intracellular delivery of fluorescently labeled siRNA in THP-1 and Hut78 cells without (top) and with (bottom) Leu-Fect B; red indicates siRNA localization. **(Top):** STAT5A mRNA knockdown in PBMCs following transfection with control or STAT5A-specific siRNA using Leu-Fect B (fold change relative to ACTB). STAT5A mRNA knockdown in RS4;11 cells under the same conditions. Relative cell viability of A549 cells 48 hours after transfection with control, CDC20, STAT5, or survivin siRNA using Leu-Fect B.

## | Benefits of Leu-Fect B

- High transfection efficiency in the presence of serum-containing medium.
- Simple protocol. No need to change tissue culture medium during transfection
- Minimal toxicity compared to other commercial transfection reagents, leading to better silencing.
- Non-integrating transfection reagent eliminates adverse effects due to host genome integration.

## | Transfection Protocol

The following procedure is recommended for preparation of siRNA nanoparticles with **Leu-Fect B** and subsequent transfection of suspension-growing cells. Please ensure all reagents are at the room temperature for the procedures.

- Recommended cell suspension is 100 to 150 x $10^3$ cells/mL. Cell suspension can be prepared at the desired concentration before or during the incubation of complexes.
- The final recommended concentration for siRNA is 40 to 60 nM and for **Leu-Fect B** is 3.15 to 4.7 µg/mL, corresponding to siRNA: **Leu-Fect B** ratio of 1:7.5. We recommend the siRNA and **Leu-Fect A** concentrations to be optimized for individual cell types.
- Recommended amounts of siRNA and **Leu-Fect B** are shown in the Table below for different multiwell plates. The amount shown is for a single well, assuming 10 µM siRNA (0.14 µg/mL) and 1 mg/mL **Leu-Fect B** stock solutions to get final siRNA concentration 60 nM. Once the plate format is selected, complex volumes should be adjusted based on the number of replicates.
- We recommend preparation of 10% excess volume to account for any possible loss due to pipetting.
- RPMI-1640 (or equivalent) medium without antibiotics or serum is recommended for complex preparation.
- Other oligonucleotides (such as microRNA) could be substituted for siRNA for delivery purposes

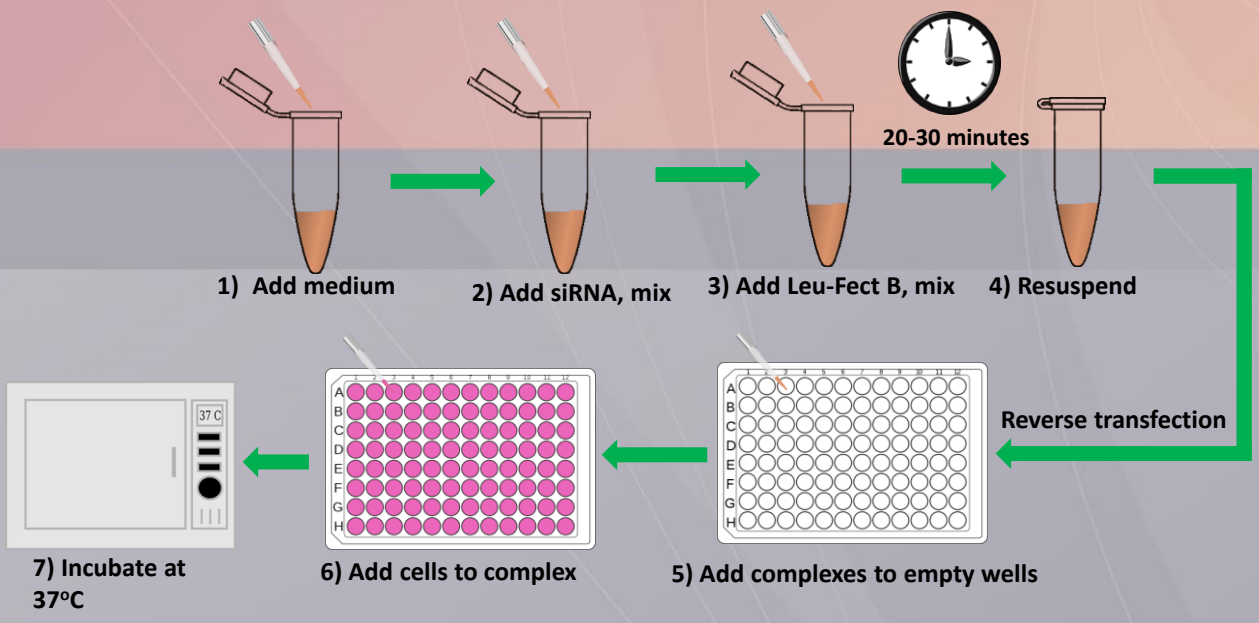
| Plate Format | Medium (µL) | siRNA (µL) | Leu-Fect B (µL) | Total Complexes (µL) | Culture Volume (µL) |
|---|---|---|---|---|---|
| **96-well** | 8.77 | 0.6 | 0.63 | 10.0 | 90.0 |
| **48-well** | 16.31 | 1.8 | 1.89 | 20.0 | 280.0 |
| **24-well** | 42.62 | 3.6 | 3.78 | 50.0 | 550.0 |
| **6-well** | 263.1 | 18.0 | 18.9 | 300.0 | 2700.0 |

Recommended Volumes. * Assuming 0.14 µg/µL (10 µM) siRNA and 1 µg/µL **Leu-Fect B** solutions. This volume will give final siRNA concentration of 60 nM and siRNA: **Leu-Fect B** ratio of 1:7.5

## | Procedure

1. Add the desired volume of medium to 1.5 mL Eppendorf microcentrifuge) tubes.
2. Add appropriate volume of siRNA solution to the medium in tubes and mix.
3. Add undiluted **Leu-Fect B** solution to the siRNA solution in medium. Vortex gently for 3 sec and incubate for 20-30 min at room temperature.
4. Re-suspend the siRNA complexes in solution using a pipette at the end of incubation.
5. Add complexes to the empty wells and ensure even distribution – manually shake plates gently if necessary.
6. Carefully add the cell suspension on top of the complexes. Gently tap plate for mixing.
7. Incubate the plate under conditions suitable for cell culture and sample cells at desired times for analysis.

## | Graphical Procedure for complex preparation and transfection




## | References


- Gul-Uludag, et al. Leukemia Research (2014) 38: 1299-1308.
- Landry, et al. J. Controlled Release (2016) 224:8-21.
- Valencia-Serna et al. J. Controlled Release (2013) 172: 495-503.