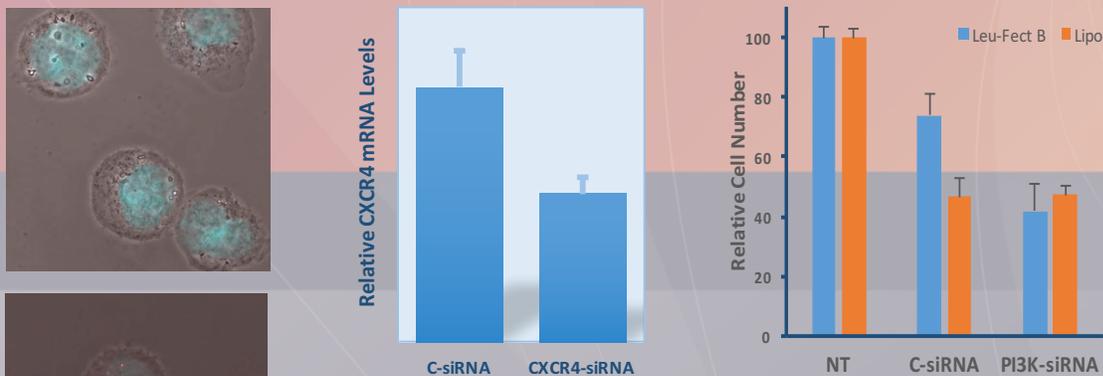


tailored transfection reagent: Leu-Fect B

PRODUCT NUMBERS: 40-10 and 40-20	SIZE: 0.75 and 1.5 mL	CONCENTRATION: 1 mg/mL	STORAGE: 4 °C
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Product Description

Leu-Fect B is a highly effective transfection reagent for attachment independent (suspension-growing) cells. **Leu-Fect B** is a synthetic amphiphilic polymer that is specifically tailored for siRNA delivery. It is capable of undergoing multivalent interactions with siRNA and encapsulating co-incubated siRNA molecules into ~100 nm particles with a net positive charge. The complexation between the siRNA 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, formulation of **Leu-Fect B** with siRNA may need to be optimized for specific cell types.



Transfecting THP-1 and Hut78 cells using Leu-Fect B. (Left) Intracellular delivery of fluorescently-labelled siRNA in the absence (top) and presence of **Leu-Fect B** (bottom; red: siRNA). (Middle): CXCR4 silencing in THP-1 cells after control (scrambled) and CXCR4 specific siRNA delivery by **Leu-Fect B**. mRNA levels of CXCR4 was detected by digital droplet PCR 2 days after transfection. (Right) Inhibition of Hut78 cell growth after control and PI3K-specific siRNA delivery by **Leu-Fect B** and a leading lipofection reagent.

| 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 10³ cells/mL. Cell suspension can be prepared at the desired concentration before or during the incubation of complexes.

- Recommended amounts of siRNA and **Leu-Fect B** reagent are shown in the Table below for different multiwell plates. The final recommended concentrations of siRNA and **Leu-Fect B** are 0.84 $\mu\text{g}/\text{mL}$ and 10 $\mu\text{g}/\text{mL}$, respectively, corresponding to siRNA:**Leu-Fect B** ratio of 1:12 and siRNA concentration of 60 nM. We recommend the siRNA and **Leu-Fect B** concentrations to be optimized for individual cell types. Suggested ranges for optimization are 0.6 to 1.2 $\mu\text{g}/\text{mL}$ for the siRNA and 6 to 15 $\mu\text{g}/\text{mL}$ for the **Leu-Fect B**. The amounts shown below are for a single well, assuming 10 μM siRNA and 1 mg/mL **Leu-Fect B** stock solutions. Once the plate format is selected, complex volumes should be adjusted based on 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.

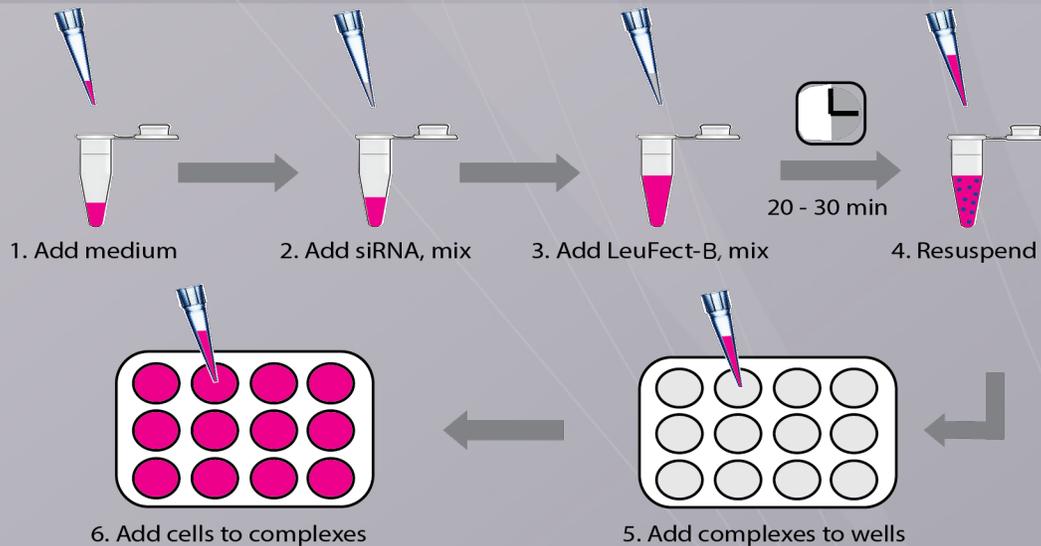
Plate Format	Medium (μL)	siRNA (μL)*	Leu-Fect-B (μL)*	Cell Suspension (μL)
96-well	50	0.9	1.5	100
48-well	100	2.4	4	300
24-well	200	4.8	8.1	600
6-well	500	12	20.2	1500

Recommended Volumes. * Assuming 0.14 $\mu\text{g}/\mu\text{L}$ (10 μM) siRNA and 1 $\mu\text{g}/\mu\text{L}$ **Leu-Fect-B** solutions.

Procedure

- Add the desired volume of medium to 1.5 mL plastic (microcentrifuge) tubes.
- Add appropriate volume of siRNA solution to the medium in tubes and vortex gently for 3 sec.
- 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.
- Re-suspend the siRNA complexes in solution using a pipette at the end of incubation.
- Add complexes to the empty wells and ensure even distribution – manually shake plates gently if necessary.
- Carefully add the cell suspension on top of the complexes. Gently tap plate for mixing.
- Incubate the plate under conditions suitable for cell culture and sample cells at desired times for analysis.

Graphical Procedure



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.