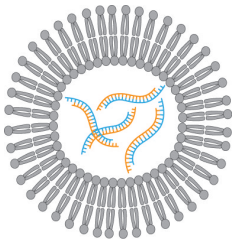


Membrane fusion is a highly efficient method for transfecting various molecules and particles into mammalian cells, even into sensitive and primary cells. The Fuse-It reagents are cargo-specific liposomal carriers that attach and rapidly fuse with plasma membranes in a physiochemical-driven manner, thus releasing their cargo into the cytoplasm of the target cell. Membrane fusion is an effective tool for functional studies, which works independently of biological processes such as endocytosis, pinocytosis, or specific receptor binding.

Overview

Fuse-It-siRNA is a proprietary formulation reagent created for the transfection of small interfering RNA (siRNA) into the cytoplasm of mammalian cells. Requiring only short incubation times of up to twenty minutes, the transfer of siRNA is highly biocompatible and non-toxic, which results in high transfer efficiency without influencing the cell viability. Due to siRNA transfer directly into the cytoplasm, lysosomal degradation and the use of lysosome function-blocking molecules are completely omitted, leading to rapid and immediate degradation of the target mRNA.

Specifications

Specifications of Fuse-It-siRNA	
Formulation	Proprietary lipids
Concentration	6 mM
Fluorescence properties	
$Ex._{max}$ / $Em._{max}$	750/780 nm (see Figure 1)
Quality Control	See Certificate of Analysis (on request)

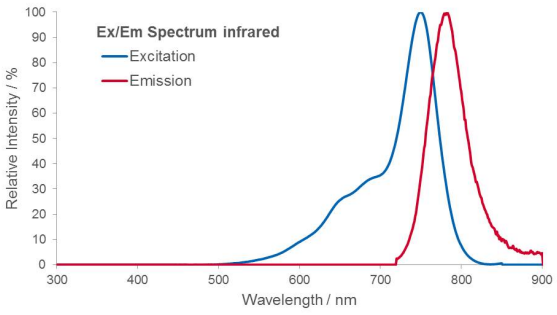


Figure 1: Spectrum of Fuse-It-siRNA fusion control dye.

Shipping and Storage

Packaging and Storage	
Shipping conditions	Ambient temperature *
Storage conditions	–20°C
Shelf life	Under proper storage conditions as indicated on vial
	3 months after opening

*Shipped with additional ice pack to assure temperature below 25°C.

Kit Contents

Fuse-It-siRNA comes as a kit containing the following components:

- Fusogenic Solution (FS): 2 vials, ready-to-use
- Neutralization Buffer (NB): 2 vials, ready-to-use

Note:

FS is solubilized in a low osmotic buffer (20 mM HEPES, pH 7.4). Aliquoting of FS or NB is not necessary.

Important Guidelines

- Fuse-It-siRNA is optimized for the transfer of RNA < 100 nt, e.g. siRNA. It is not recommended using Fuse-It-siRNA for the transfer of RNA > 100 nt, e.g. mRNA. In this case, use Fuse-It-mRNA (Cat. No. 60500, 60501) instead.

- Due to the infrared (IR) control dye, the fusion process can be visualized directly after fusion. Make sure to use the appropriate IR filter sets and an IR-sensitive camera. The IR fluorescence signal of the liposomal carriers can also be applied for cell sorting using flow cytometry.
- Use high-quality, thin bottom cell culture materials to achieve the best imaging results (e.g., ibidi's µ–Slides and µ–Dishes).

Protocol: Transfection of siRNA Into Mammalian Cells

Material Required but Not Supplied

- Ultrasonic bath (effective power of at least 40 W)
  Note: Before starting, cool the ultrasonic bath to 4°C, if necessary add ice to the water.
- siRNA (10–60 µM) in RNase-free buffer. Find the necessary amount for various culture formats in Table 1.
- RNase-free micro reaction tubes
- 1× PBS (pH 7.0–8.0; osmolarity 250–320 mOs/kg)
  Note: Divalent ions do not influence the fusion process.

Day 0

Preparation of Adherent Cells

- One day before transfection, seed the adherent cells and incubate them under standard conditions (typically 37°C and 5% CO_2). The cells should be 70% –90% optically confluent at time of transfection.

Preparation of Suspension Cells

- Prepare enough cells for the experiment. A cell number of 1–3×10^5 cells is recommended for each siRNA transfection reaction.
  Note: Higher cell numbers should be used for sensitive cells to achieve a less harsh fusion reaction.

Day 1

Important Notes

- All steps should be performed on ice, unless indicated otherwise.
- The cells should be in a healthy condition at the time of transfection.
- The volumes in this protocol (marked in red) refer to one single siRNA transfection of adherent cells in a µ–Dish $^{35\ mm,\ high}$ or 1–3×10^5 suspension cells. Please calculate the respective volumes according to the experimental setup and culture format as indicated in Table 1.

Culture Format	Growth Area[+]	siRNA Amount	Neutralization Buffer (NB)	Fusogenic Solution (FS)	Transfection Mix	Culture Medium
µ–Dish $^{35\ mm,\ high}$ (ibidi)	3.5 cm^2	10–25 pmol	2–5 µl	1.25 µl	250 µl	2 ml
µ–Plate 96 Well (ibidi)	0.55 cm^2	2–4 pmol	0.4–0.8 µl	0.6 µl	100 µl	300 µl
96–well	0.3 cm^2	1–3 pmol	0.2–0.6 µl	0.4 µl	70 µl	300 µl
48–well	0.75 cm^2	2–5 pmol	0.4–1 µl	0.7 µl	150 µl	750 µl
24–well	1.9 cm^2	5–10 pmol	1–2 µl	1.25 µl	300 µl	1 ml
12–well	3.8 cm^2	10–25 pmol	2–5 µl	2.5 µl	550 µl	2 ml
6–well	9.5 cm^2	25–60 pmol	5–12 µl	5 µl	1000 µl	3 ml

[+]Growth area varies depending on the manufacturer.

Table 1: Volumes for siRNA transfection of adherent cells in different culture vessels. The transfection conditions must be adjusted in proportion to the relative surface area. Volumes and amounts are given on a per-well-basis.

Before Starting

- Thaw Neutralization Buffer (NB) and Fusogenic Solution (FS) at room temperature. Then place the vials on ice.
- Cool ultrasonic bath to 4°C. If necessary add ice to the water.
- Heat 2 ml culture medium to 37°C.
- Cool 1× PBS to 4°C.

Preparation of Fusogenic Solution

1. Dilute the siRNA to a final concentration of 10 µM in RNase-free water. Mix by pipetting up and down several times.
   Note: A 10 µM siRNA solution is equivalent to 10 pmol/µl. The siRNA concentration can range from 10 to 60 µM, according to the experimental setup and the required siRNA amount.
2. Briefly vortex the Neutralization Buffer (NB). Into one tube, add 2 µl of NB and 10 pmol of siRNA (e.g. 1 µl of a 10 µM siRNA solution) and mix by pipetting up and down several times. This is the neutralized siRNA solution.
   Note: Always keep the same ratio: 2 µl NB : 10 pmol siRNA. Adjust according to the experimental setup and the required siRNA amount, as indicated in Table 1.
3. Incubate neutralized siRNA for 10 minutes at **room temperature**. In the meantime, continue with step 4.
4. Vortex the Fusogenic Solution (FS), which contains the liposomal carriers, until the solution is homogeneous. If necessary, mix by pipetting up and down several times.
   Note: The Fuse-It-siRNA solution needs to be a homogeneous solution at every step. Aggregates can affect the fusion efficiency!
5. In one new tube, add 1.25 µl of the FS and sonicate in a cooled ultrasonic bath for 5 minutes.
6. Transfer the sonicated FS to the tube containing the neutralized siRNA solution and mix by pipetting up and down several times. This is the non-diluted transfection solution.
7. Sonicate in a cooled ultrasonic bath for 5 minutes.
8. Add cooled 1× PBS to the non-diluted transfection solution to a final volume of 250 µl and vortex briefly.
9. Sonicate in a cooled ultrasonic bath for 5 minutes.
10. For adherent cells, continue with ”A) Transfection of Adherent Cells”, for suspension cells, continue with ”B) Transfection of Cells in Suspension”.

A) Transfection of Adherent Cells

1. Remove the culture medium and add the transfection solution drop-wise onto the cells.
2. Incubate for 5–20 minutes at 37°C until the cells start to detach.
   Note: Reaching 37°C is critical for optimal fusion efficiency! Observe the cell morphology every 5 minutes to determine the optimal reaction time for the cells. The reaction should be stopped before signs of cell detachment are observed.
3. Remove the transfection solution and add fresh culture medium (equilibrated to 37°C) to the cells to stop the fusion process.
4. Incubate the cells under standard cell culture conditions (typically 37°C and 5% CO_2) before analysis of gene knockdown. The timepoint of analysis depends on the experimental setup, typically 24–48 hours after transfection.

B) Transfection of Cells in Suspension

1. Centrifuge 1–3×10^5 cells at 200 ×g for 5 minutes and discard the supernatant.
   Note: For certain cell types, a higher centrifugation speed might be necessary for pelleting.
2. Resuspend the cell pellet in 50 µl of fresh cell culture medium containing 10% serum.
   Note: The cell culture medium must contain 10% serum! The serum proteins protect the cells in suspension to achieve a less harsh fusion reaction.
3. Add the transfection solution to the resuspended cells and vortex briefly.
4. Incubate for 1–5 minutes at room temperature.
   Note: Fusion is very efficient when using cells in suspension. Therefore, only a short incubation time is needed. The optimal incubation time has to be determined for each cell type.
5. Pellet the cells at 600–800 ×g for 3 minutes.
   Note: 600–800 ×g is required for pelleting, depending on the cell type. At lower speed, the cells largely remain in the supernatant due to liposomal fusion.
6. Remove the transfection solution and resuspend the cells in fresh culture medium.
7. Incubate the cells under standard cell culture conditions (typically 37°C and 5% CO_2) before analysis of gene knockdown. The timepoint of analysis depends on the experimental setup, typically 24–48 hours after transfection.

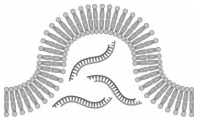
Troubleshooting Guide

Problem	Possible Cause	Recommendation
Cell viability is reduced after fusion and/or cells detach from the surface during or after fusion.	Incubation of sensitive cells in 1× PBS caused cell stress.	To reduce cell stress, cell culture medium can be used for the dilution of the transfection solution instead of 1× PBS. **Note:** For adherent cells, the use of a serum-containing medium may reduce the fusion efficiency.
	Cells were stressed or generally showed a weak adherence to the surface.	Reduce the incubation time with the transfection solution. A substrate coating may be helpful for sensitive cells.
	Cell density was too low.	Increase cell number. Optical confluence should range from 70% to 90%. The number of cells per transfection in suspension can be increased up to 1×10^6 cells.
	siRNA : Fuse-It ratio was not optimal for the used cell type.	Optimize the siRNA : Fuse-It ratio for the used cell type.
	siRNA was of poor quality or contained endotoxins.	Ensure that the stock solution of the siRNA is of high quality and reconstituted in RNase-free and endotoxin-free buffer, according to the manufacturer´s recommendations.
	Transfection solution was not homogeneously added, resulting in accumulation of the transfection solution.	Add the transfection solution dropwise onto the adherent cells for a homogenous distribution.
Fusion efficiency, as indicated by the included infrared control dye, was low.	Remaining serum proteins from the culture medium reduced the fusion efficiency.	Wash the cells with 1× PBS prior to adding the transfection solution to the cells.
	Incubation time with transfection solution was too short.	Increase the incubation time to a maximum of 20 minutes for the fusion of adherent cells or to a maximum of 5 minutes for the fusion of suspension cells, respectively.
	Transfection solution was not homogeneously distributed during incubation.	Gentle motion during incubation will improve fusion efficiency.
	Cell density was not optimal.	For optimal fusion efficiency, optical confluence should range from 70% to 90%. If a lower cell density is required, adherent cells can also be transfected in suspension. This might enhance the fusion efficiency.

	Fuse-It reagents were stored improperly or warmed up during sonication.	Store Fuse-It reagents at –20°C. Always work on ice, if not indicated otherwise. Cool the ultrasonic bath to 4°C. If necessary, add ice to the water.
Despite high fusion efficiency, siRNA transfection efficiency was too low.	Low amount of siRNA was transferred.	Increase the initial siRNA amount, but keep the same ratio 10 pmol siRNA : 2 µl NB. **Note:** A more than fivefold increase of siRNA can affect cell viability.
	Standard siRNA : FS ratio was not optimal for the cell type.	Increase the initial FS volume. **Note:** A more than twofold increase of FS can affect cell viability.
	Incubation period of siRNA and NB was too short.	We recommend a neutralizing incubation time of 10 minutes. To optimize this process, incubate for up to 20 minutes.
	Degradation or poor quality of siRNA.	Ensure that the stock solution of the siRNA is of high quality and reconstituted in RNase-free and endotoxin-free buffer, according to the manufacturer´s recommendations.
Transfection results are not reproducible.	Transfection was conducted using different cell confluencies.	Keep cell confluency constant throughout all experiments.
	Cells changed their morphology or behavior over time.	Transfection efficiency is dependent on passage number. Use similar passage numbers for each transfection.
	Wrong amount of siRNA, NB, or FS were used, due to pipetting errors.	When pipetting low volumes of siRNA, NB, or FS, it is recommended to prepare 1:10 dilutions in RNase-free water. **Note:** FS has to be sonicated once before dilution.
Knockdown was too low.	siRNA worked not efficient enough for the target mRNA.	Use a different siRNA, or a siRNA pool to knockdown the target mRNA.
	Knockdown was low, due to the long-term stability of the protein.	Daily repeat of the fusion is possible, due to the high biocompatibility and low cytotoxicity of Fuse-It-siRNA.

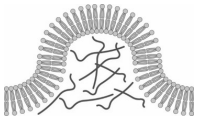
Ordering Information

Fuse-It-siRNA: Transfer of siRNA



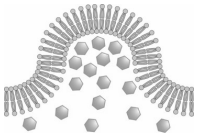
Cat. No.	Description	Amount
60510	**Fuse-It-siRNA, infrared fluorescent:** ready to use, 6 mM	2 × 150 µl
60511	**Fuse-It-siRNA, infrared fluorescent:** ready to use, 6 mM	2 × 300 µl

Fuse-It-mRNA: Transfer of mRNA



Cat. No.	Description		Amount
60505	**Fuse-It-mRNA**	**infrared fluorescent:** ready to use, 6 mM	2 × 150 µl
60506	**Fuse-It-mRNA**	**infrared fluorescent:** ready to use, 6 mM	2 × 300 µl

Fuse-It-P: Transport of Proteins



Cat. No.	Description	Amount
60220	**Fuse-It-P, infrared fluorescent:** lyophilized, 3 mM	for 100 µl solution
60221	**Fuse-It-P, infrared fluorescent:** lyophilized, 3 mM	for 4 ×25 µl solution
60222	**Fuse-It-P, infrared fluorescent:** lyophilized, 3 mM	for 400 µl solution
60223	**Fuse-It-P, infrared fluorescent:** lyophilized, 3 mM	for 4 ×100 µl solution

For research use only!

Further technical specifications can be found at www.beniag.com. For questions and suggestions please contact us by e-mail info@beniag.com. All products are developed and produced in Germany.

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