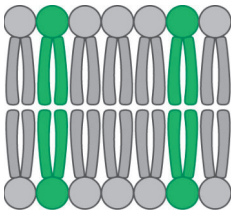


Membrane fusion is a novel and highly superior method for incorporating various molecules and particles into mammalian cells. Cargo-specific liposomal carriers are able to attach and rapidly fuse with plasma membranes in a physicochemical-driven manner, thus releasing their cargo into the cytoplasm of the target cell. Membrane fusion is, therefore, an effective strategy for functional studies and therapeutic approaches.
The new ibidi Fuse-It reagents use this mechanism to fuse with mammalian cell surfaces immediately upon contact. Using this novel technique makes the transfer of molecules possible and independent of biological processes such as endocytosis, pinocytosis, or specific receptor binding.

Overview

Fuse-It-L is a proprietary formulation reagent for transferring lipids and other amphipathic molecules into the plasma membrane of a wide range of eukaryotic cells—within minutes.
The reagent-molecule complexes can be added to adherent cells, as well as to cells in suspension, independent from medium conditions. In addition, transferred lipids are instantly active inside the plasma membrane and, after fusion, cells can immediately be used for further analysis.

Specifications

Specifications of Fuse-It-L	
Formulation	Proprietary lipids
Concentration	3 mM
Fluorescence properties	
$Ex._{max}$ / $Em._{max}$	750/780 nm (see Figure 1)
Quality Control	See Certificate of Analysis (on request)

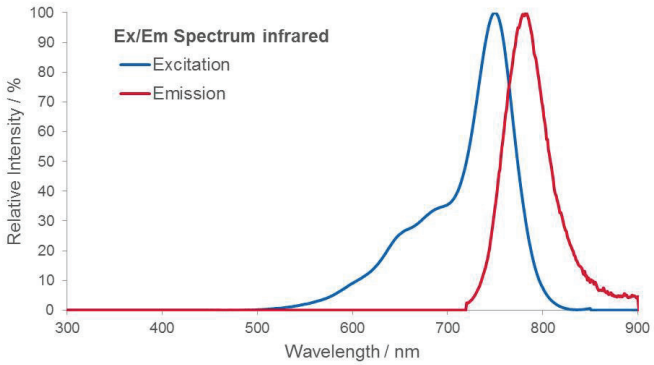


Figure 1: Spectrum of Fuse-It-L fusion control dye.

Shipping and Storage

Packaging and Storage	
Shipping conditions	Ambient temperature *
Storage conditions	–20°C
Shelf life (lyophilized)	Under proper storage conditions as indicated on vial

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

Additional Material Required

- Lipid or amphipathic molecule
- Organic solvent resistent pipette, e.g. glass
- HEPES buffer (pH 7.0–8.0; 20 mM)
- 1× PBS (pH 7.0–8.0; osmolarity 250–350 mOs/kg)
- Ultrasonic bath
  Note: An effective power of 50 to 200 W, and a frequency of 35 to 50 kHz are necessary. The use of an ultrasonic probe is also possible, but the probe must be cooled thoroughly during sonication in a pulsed mode.

Important Guidelines

- Use high quality lipids and amphipathic molecules dissolved in chloroform or alcohol.
- Two or more amphipathic molecules can be transferred by co-fusion in parallel as long as their total amount does not exceed the indicated maximal amount.
- Evaporation of the solvent should take place under vacuum or reduced pressure. The use of a desiccator connected to a vaccum pump (water jet pump or membrane pump) is recommended.
- After resuspension in HEPES buffer, Fuse-It-L should not be stored. Any added molecules might affect the stability of Fuse-It-L. Also, the overall stability of molecule of interest may vary.
- For the first use of Fuse-It-L, we recommend using the protocol on page 2. For further optimization, see page 5.
- Due to an infrared (IR) dye, the fusion process can be visualized directly after fusion using fluorescence microscopy. 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 used for cell sorting when 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

The protocols are designed for the incorporation of lipids into cell membranes in an open format using:

• Fuse-It-L for 25 µl	60211
⋆ Fuse-It-L for 100 µl	60210 and 60213
△ Fuse-It-L for 400 µl	60212

The appropriate volumes for different formats are listed on page 6.

Note:

After resuspension the Fuse-It-L solution needs to be a homogeneous solution at every step. Small aggregates can affect the fusion efficiency.

Cell Preparation for Adherent Cells

One day before the experiment, seed the cells so as to reach >70% optical confluence at the time of fusion.

Fusion of Adherent Cells

To fuse adherent cells in a µ–Dish $^{35mm,\ high}$ (growth area 3.5 cm^2) with the liposomes of Fuse-It-L, please follow these steps:

1. Add a maximum of • 4 nmol, or ⋆ 10 nmol, or △ 40 nmol of the lipid or amphipathic molecule solved in chloroform or alcohol to one Fuse-It-L vial and vortex until the solution is homogeneous. If necessary, mix by pipetting.
2. Evaporate the solvent entirely under vacuum or reduced pressure. At least • 30 min for 25 µl, or ⋆ 1 h for 100 µl, or △ 2 h for 400 µl solvent.
3. Add • 25 µl, or ⋆ 100 µl, or △ 400 µl of 20 mM HEPES buffer and vortex until the solution is homogeneous. If necessary, mix by pipetting.
4. Sonicate the mixture in a standard ultrasonic bath for 10–20 minutes at room temperature, or lower.[1]
5. Add 1× PBS to 10 µl of the fusogenic mixture to make a final volume of 1000 µl, and mix thoroughly.
   Note: Keep all components below room temperature!
6. Sonicate the dilution in a standard ultrasonic bath for 5 minutes at room temperature, or lower.[1]
7. Remove the culture medium and add the diluted fusogenic mixture drop-wise onto the cells.
8. Incubate for 2 minutes at 37°C.
   Note: Reaching 37°C is very important!
9. Replace the fusogenic mixture with fresh culture medium to stop the fusion.
10. **Optionally** wash the cells with 1× PBS or fresh culture medium.
11. After fusion, the cells are available for further experiments.

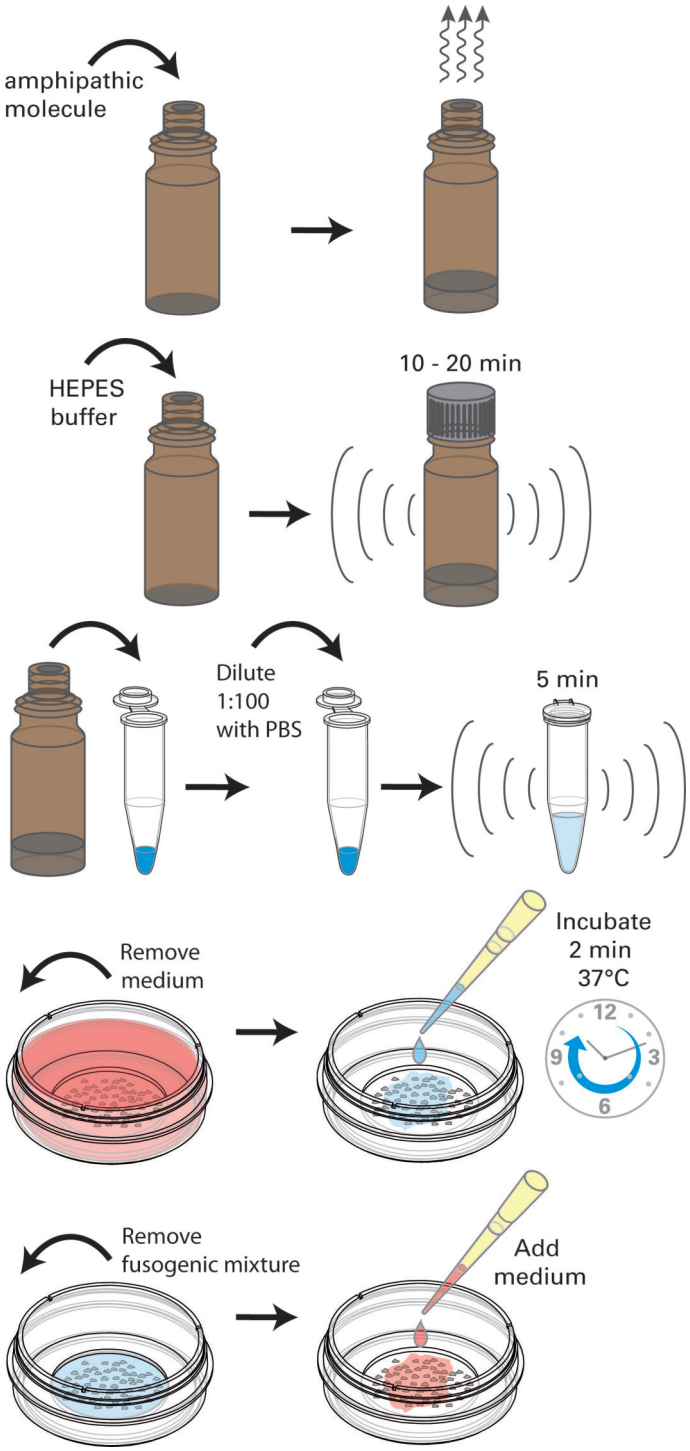


Figure 2: Schematic overview of the Fuse-It-L system with adherent cells.

[1] Make sure the water bath temperature remains below 25°C throughout the entire sonication! If necessary, add ice.

Cell Preparation for Suspension Cells

Use a cell number of 1–3× 10^5 cells on the day of fusion. The number of cells per fusion can be increased up to 1× 10^6 cells.

Fusion of Suspension Cells

To fuse cells in suspension in a µ–Dish $^{35mm, high}$ (growth area 3.5 cm^2) with the liposomes of Fuse-It-L, please follow these steps:

1. Add a maximum of • 4 nmol, or ⋆ 10 nmol, or △ 40 nmol of the lipid or amphipathic molecule solved in chloroform or alcohol to one Fuse-It-L vial and vortex until the solution is homogeneous. If necessary, mix by pipetting.
2. Evaporate the solvent entirely under vacuum or reduced pressure. At least • 30 min for 25 µl, or ⋆ 1 h for 100 µl, or △ 2 h for 400 µl solvent.
3. Add • 25 µl, or ⋆ 100 µl, or △ 400 µl of 20 mM HEPES buffer and vortex until the solution is homogeneous. If necessary, mix by pipetting.
4. Sonicate the mixture in a standard ultrasonic bath for 10–20 minutes at room temperature, or lower.[1]
5. Add 1× PBS to 10 µl of the fusogenic mixture to make a final volume of 1000 µl, and mix thoroughly.
   Note: Keep all components below room temperature!
6. Sonicate the dilution in a standard ultrasonic bath for 5 minutes at room temperature, or lower.[1]
7. Centrifuge 1–3× 10^5 cells and discard the supernatant.
8. Resuspend the cell pellet in the diluted fusogenic mixture.
9. Incubate cells in suspension for 1–3 minutes at room temperature.
10. Stop fusion by adding 1 ml (1 volume) of cell culture medium.
11. Centrifuge cells at an elevated speed (600 to 800 ×g) for 3 minutes.
    Note: At normal speed, the cells largely remain in supernatant due to liposomal fusion.
12. Resuspend the cells in fresh culture medium.
13. After fusion, the cells are available for further experiments.

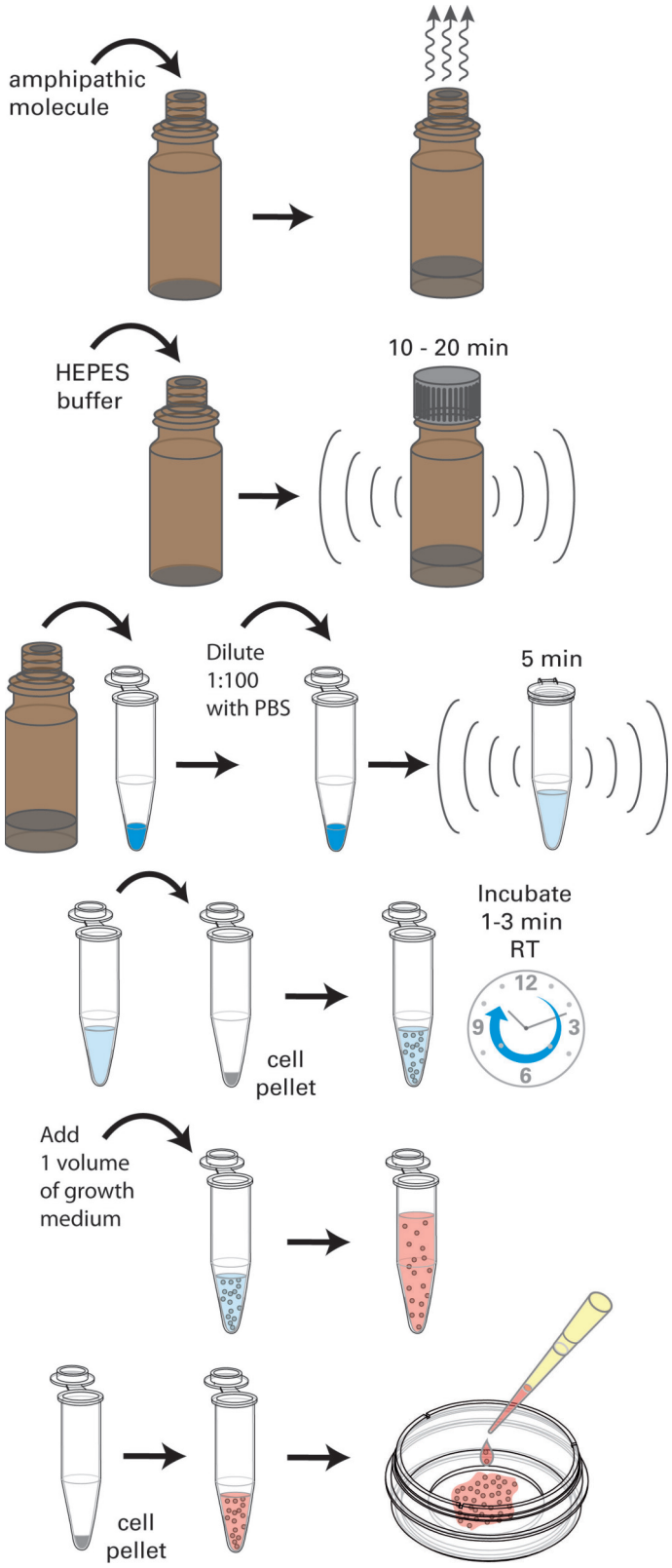


Figure 3: Schematic overview of the Fuse-It-L system with suspension cells.

Optimization of the Fusion Process

Results may vary slightly between cell types. You can identify the optimal condition for each cell type by adjusting several parameters.

- Increasing efficiency:
  - Vary the dilution of the fusogenic mixture between 1:50 and 1:100 in 1× PBS.
  - Vary the incubation time between 1-15 minutes for the fusion of adherent cells; 1-3 minutes for the fusion of suspension cells.
  - Gentle motion during incubation will improve fusion efficiency.
- The complete resuspension of the lyophilized reagent is highly relevant for successful fusion. For • Fuse-It-L for 25 µl, slightly open the screw cap before vortexing so the inner vial can move freely. If necessary, mix by pipetting.
- The amount of Fuse-It-L required for successful fusion may vary slightly depending on the cell type and passage number.
- Instead of using 1× PBS, cell culture medium can also be used for the dilution of the fusogenic mixture.
  Note: The use of a medium containing serum may reduce the fusion efficiency.
- If cell detachment is observed during the fusion process, the incubation time should be reduced.
- A coating could be helpful for sensitive cells.
- Depending on cell type, cells might re-adhere slightly slower after fusion in suspension. If necessary, use the protocol for fusion of adherent cells.

Scaling Up or Down

Adherent Cells

To fuse adherent cells in different tissue culture formats, the fusion conditions must be adjusted in proportion to the relative surface area, as shown in the following table. Volumes and amounts are given on a per well basis.

Culture Vessel	Growth Area*	Fusogenic Solution	Final Volume
µ–Dish $^{35\ mm,\ high}$ (ibidi)	3.5 cm^2	10.0 µl	1000 µl
µ–Slide 8 Well (ibidi)	1.0 cm^2	3.0 µl	300 µl
µ–Plate 96 Well (ibidi)	0.55 cm^2	2.0 µl	200 µl
96–well	0.3 cm^2	1.4 µl	140 µl
48–well	0.75 cm^2	3.0 µl	300 µl
24–well	1.9 cm^2	6.0 µl	600 µl
12–well	3.8 cm^2	10.0 µl	1000 µl
6–well	9.5 cm^2	20.0 µl	2000 µl

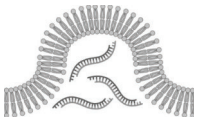
*Growth area varies depending on the manufacturer.

Suspension Cells

To fuse cells in suspension in different tissue culture formats, the volumes and amounts increase linearly with the cell number.

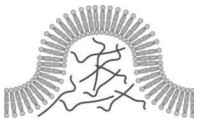
Ordering Information

Fuse-It-siRNA: Transfer of siRNA



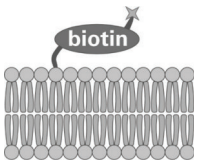
Cat. No.	Description	Amount
60510	**Fuse-It-siRNA, infrared fluorescent:** ready to use, 6 mM	2 × 150 µl
60511	**Fuse-It-siRNA, infrared fluorescent:** ready to use, 6 mM	2 × 300 µl

Fuse-It-mRNA: Transfer of mRNA



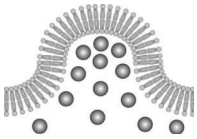
Cat. No.	Description	Amount
60500	**Fuse-It-mRNA, infrared fluorescent:** ready to use, 6 mM	2 × 150 µl
60501	**Fuse-It-mRNA, infrared fluorescent:** ready to use, 6 mM	2 × 300 µl

Fuse-It-B: Membrane Biotinylation



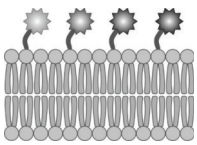
Cat. No.	Description	Amount
60320	**Fuse-It-B^{green}, green fluorescent:** ready to use, 3 mM	100 µl
60321	**Fuse-It-B^{green}, green fluorescent:** ready to use, 3 mM	400 µl
60322	**Fuse-It-B^{red}, red fluorescent:** ready to use, 3 mM	100 µl
60323	**Fuse-It-B^{red}, red fluorescent:** ready to use, 3 mM	400 µl

Fuse-It-Beads: Transport of Beads



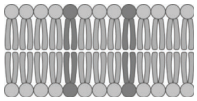
Cat. No.	Description	Amount
60420	**Fuse-It-Beads, infrared fluorescent:** ready to use, 3 mM	100 µl
60421	**Fuse-It-Beads, infrared fluorescent:** ready to use, 3 mM	400 µl

Fuse-It-Color: Membrane Staining



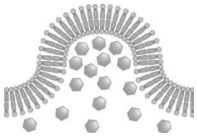
Cat. No.	Description	Amount
60200	**Fuse-It^{green}, green fluorescent:** ready to use, 3 mM	100 µl
60201	**Fuse-It^{green}, green fluorescent:** ready to use, 3 mM	400 µl
60202	**Fuse-It^{red}, red fluorescent:** ready to use, 3 mM	100 µl
60203	**Fuse-It^{red}, red fluorescent:** ready to use, 3 mM	400 µl
60204	**Fuse-It^{dred}, dark red fluorescent:** ready to use, 3 mM	100 µl
60205	**Fuse-It^{dred}, dark red fluorescent:** ready to use, 3 mM	400 µl
60206	**Fuse-It^{IR}, infrared fluorescent:** ready to use, 3 mM	100 µl
60207	**Fuse-It^{IR}, infrared fluorescent:** ready to use, 3 mM	400 µl

Fuse-It-L: Lipid Insertion



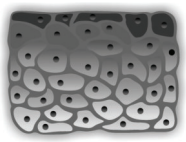
Cat. No.	Description	Amount
60210	**Fuse-It-L, infrared fluorescent:** lyophilized, 3 mM	for 100 µl solution
60211	**Fuse-It-L, infrared fluorescent:** lyophilized, 3 mM	for 4 ×25 µl solution
60212	**Fuse-It-L, infrared fluorescent:** lyophilized, 3 mM	for 400 µl solution
60213	**Fuse-It-L, infrared fluorescent:** lyophilized, 3 mM	for 4 ×100 µl solution

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

Fuse-It-T: Tissue Staining



Cat. No.	Description	Amount
60260	**Fuse-It-T, red fluorescent:** ready to use, 3 mM	100 µl
60261	**Fuse-It-T, red fluorescent:** ready to use, 3 mM	400 µl

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.

beniag GmbH, Huthmacherstrasse 20, 52428 Juelich, Germany.