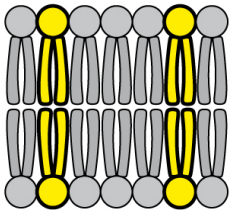

Membrane fusion is a novel and highly superior method to incorporate various molecules and particles into mammalian cells, and a strong strategy for functional studies and therapeutic approaches. Specific liposomal carriers are able to attach and instantly fuse with plasma membranes in a physicochemical-driven manner. ibidi's new Fuse-It reagents efficiently use this mechanism and fuse with mammalian cell surfaces immediately upon contact. Therefore, this novel technique makes the transfer of molecules 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-within minutes-into the plasma membrane of a wide range of eukaryotic cells. Reagent-molecule complexes can be added to adherent cells, as well as to cells in suspension, independent from medium conditions. Plus, transferred lipids are instantly active inside the plasma membrane and, after fusion, cells can immediately be used for further analysis.

Specifications

Formulation	Proprietary lipids
Concentration	3 mM
Shipping conditions	Room temperature
Storage conditions	-20°C
Shelf life (lyophilized)	Under proper storage conditions as indicated on vial.
Fluorescence properties	
$Ex._{max}$ / $Em._{max}$	750/780 nm

Additional Material Required

Lipid- or amphipathic molecule - solution
HEPES buffer
Ultrasonication bath

Important Guidelines

- Use high quality lipids and amphipathic molecules dissolved in chloroform or alcohol. Molecules sensitive to these solvents (e.g. proteins) cannot be transferred with Fuse-It-L. Please use appropriate Fuse-It products instead.
- Two or more amphipathic molecules can be transferred by co-fusion in parallel as long as their total concentration does not exceed the indicated maximal concentration.
- After resuspension in HEPES buffer, Fuse-It-L itself is stable for 2 months at 4°C, and uo to 6 months at -20°C. Any added molecules might affect the stability of Fuse-It-L. Also, the overall stability of molecule of interest may vary.
- For first time fusions, we recommend different incubation times and concentrations of the reagent for incubation with cells, in order to determine the best fusion efficiencies.
- Efficiencies can be verified directly after fusion and also be used for flow cytometric cell sorting when using the appropriate sensitive cameras or detectors (for details see specifications).
- Use high-quality, thin bottom cell culture materials to achieve the best imaging result (e.g., ibidi's µ-Slides and µ-Dishes).

Note:

Fuse-It-L is a highly effective and fast molecule transfer system. Incubation times of as short as just one minute might already be sufficient for receiving high efficiencies. Therefore, prolonged incubation times will not improve fusion efficiencies, but might instead harm the cells.

Protocol

The protocols are designed for the preparation of 100 µl of one amphipathic molecule and the use of one µ–Dish $^{35mm, high}$ (volume 1 ml, growth area 3.5 cm^2).

Cell Preparation for adherent cells

Seed cells to reach 50 – 90% confluence per µ-Dish in 1 ml of culture medium one day before fusion.

Fusion of adherent cells

Note:

You can also use trypsinized cells with the protocol for the fusion of suspension cells.

1. Add a maximum of 10 nmol of amphipathic molecule in chloroform to a single Fuse-It-L vial and mix thoroughly by vortexing until complete resuspension.
2. Evaporate the solvent entirely.
3. Resuspend the reagent-molecule complex in 100 µl of 20 mM HEPES buffer (pH 7.4) and mix thoroughly by vortexing until complete resuspension.
4. Sonicate the mixture in a standard ultrasonic bath for 10 – 20 minutes at room temperature or lower.

Note:

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

5. Dilute 5 µl* of the fusogenic mixture in 500 µl 1 × PBS by vortexing for 30 seconds.
   Note: Keep all components below room temperature!
6. Replace the culture medium of the cells with diluted fusogenic mixture.
7. Incubate for 2 minutes* at 37°C.
8. Replace the fusogenic mixture with fresh culture medium to stop fusion.
9. After fusion, the cells are immediately available for further experiments.

*For optimization of the fusion process see page 4.

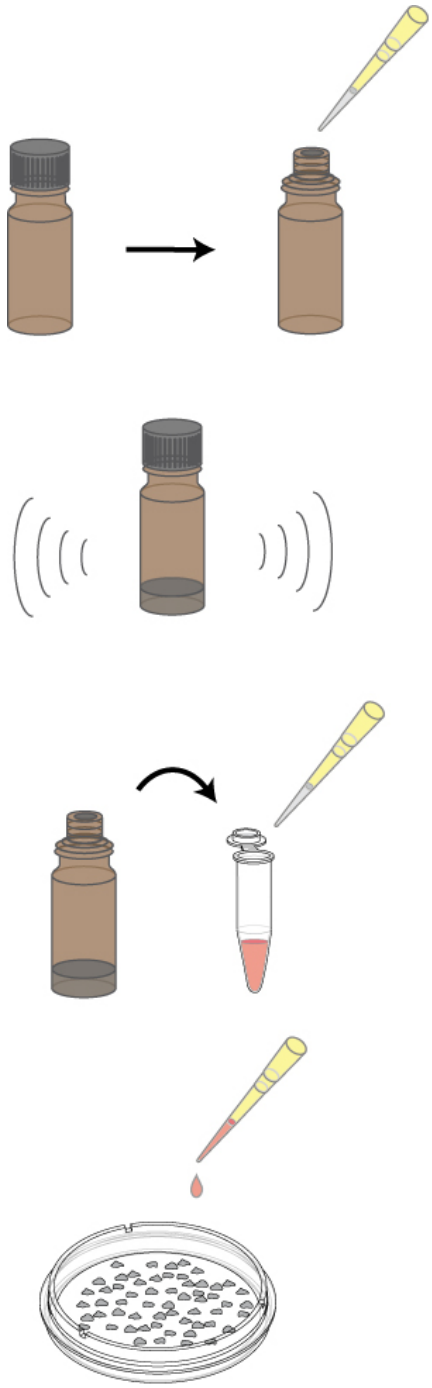

Figure 1: Schematic overview of the Fuse-It-L system with adherent cells.
Find more information on www.ibidi.com.

Cell Preparation for suspension cells

Use 1 – 3 × 10^5 cells/ml per µ-Dish on the day of fusion (the number of cells per fusion can be enhanced up to 6 × 10^6).

Fusion of suspension cells

1. Add a maximum of 10 nmol of amphipathic molecule in chloroform to a single Fuse-It-L vial and mix thoroughly by vortexing until complete resuspension.
2. Evaporate the solvent entirely.
3. Resuspend the reagent-molecule complex in 100 µl of 20 mM HEPES buffer (pH 7.4) and mix thoroughly by vortexing until complete resuspension.
4. Sonicate the mixture in a standard ultrasonic bath for 10 – 20 minutes at room temperature or lower.

Note:

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

5. Dilute 5 µl* of the fusogenic mixture in 500 µl 1 × PBS by vortexing for 30 seconds.
   Note: Keep all components below room temperature!
6. Centrifuge the cells and discard the supernatant.
7. Resuspend the pellet in the diluted fusogenic mixture.
8. Incubate cells in suspension for 1 – 3 minutes at 37°C.
9. Stop fusion by adding 1 ml 1 × PBS.
10. Centrifuge cells at an elevated speed (600 to 800 ×g).
    Note: At normal speed, the cells largely remain in supernatant due to liposomal fusion.
11. Wash cells after centrifugation with 1 × PBS, once, or resuspend them directly in fresh culture medium.
12. After fusion, the cells are immediately available for further experiments.

*For optimization of the fusion process see page 4.

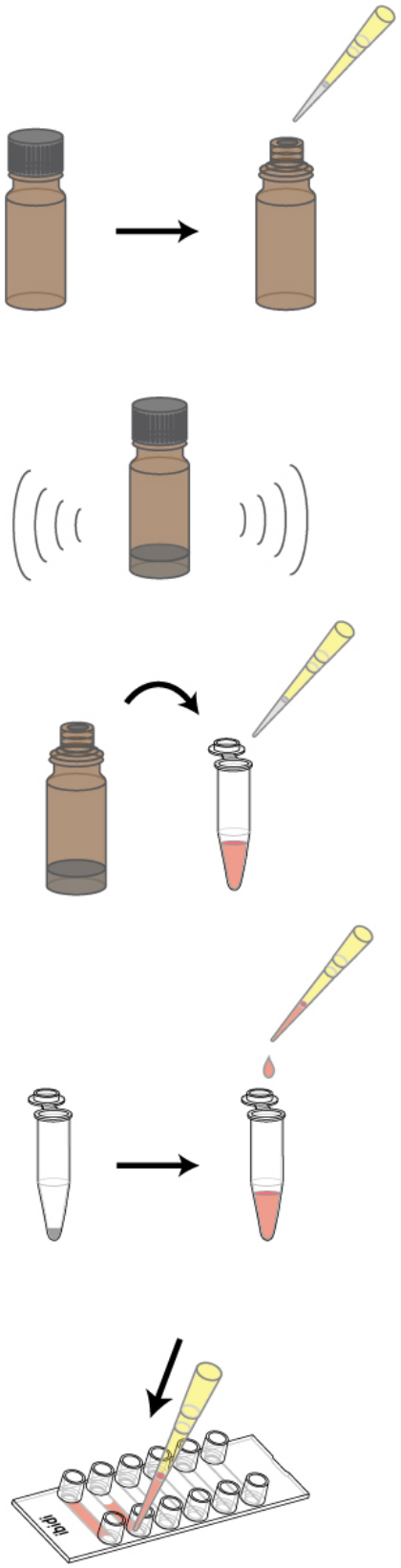

Figure 2: Schematic overview of the Fuse-It-L system with suspension cells.
Find more information on www.ibidi.com.

Optimization of the fusion process

- Results may vary slightly between cell types. If necessary, the incubation time and the volume of the fusogenic mixture can be further adjusted.
  - Vary the dilution of the fusogenic mixture between 5 – 10 µl in 500 µl 1 × PBS.
  - Vary the incubation time between 1 – 10 minutes for the fusogenic mixture on cells.
- Gentle motion during incubation improves fusion efficiency.
- Instead of using 1 × PBS, serum free cell culture medium can also be used for the dilution of the fusogenic mixture.
- Reaching 37 °C during fusion is very important.
- The amount of Fuse-It-L required for successful fusion may vary slightly depending on the cell type and passage number.
- High confluencies may be helpful, but not mandatory.
- Depending on cell type, cells might re-adhere slightly slower after fusion. If necessary, use the protocol for fusion of adherent cells.

Fuse-It-L

Ordering Number	Labeling	Fluorescence (Ex.$_{max}$ / Em.$_{max}$)	Amount	
60210	Fuse-It-L	750/780 nm	100	µl solution
60211	Fuse-It-L	750/780 nm	4 ×25	µl solution
60212	Fuse-It-L	750/780 nm	400	µl solution
60213	Fuse-It-L	750/780 nm	4 ×100	µl solution

µ–Dish 35mm, high

Ordering Number	Treatment or Coating	Characteristics
81156	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
81158	glass bottom, sterile	glass coverslip, No. 1.5H

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.