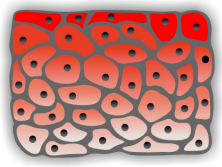

Membrane fusion is a novel and highly superior method to incorporate various molecules and particles into mammalian cells, and a strong strategy for functional studies and therapeutic approaches. Specific liposomal carriers are able to attach and instantly fuse with plasma membranes in a physicochemical-driven 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-T is a proprietary formulation reagent for stable, biocompatible plasma membrane labeling-within minutes-of thick layers of a wide range of eukaryotic cells. Reagent can be added to adherent tissue sheets, as well as to tissue sheets in suspension, independent of medium conditions. Plus, after fusion, tissue sheets can immediately be used for further analysis.

Specifications

Formulation	Proprietary lipids
Concentration	3 mM
Shipping conditions	Room temperature
Storage conditions	-20°C
Shelf life	Under proper storage conditions as indicated on vial.
Fluorescence properties	
Ex.$_{max}$/Em.$_{max}$	549/565 nm

Additional Material Required

Ultrasonication bath

Important Guidelines

- Fuse-It-T is solubilized in low osmotic buffer (20 mM HEPES, pH 7.4). After opening, the reagent itself is stable for 2 months at 4°C and 6 months at -20°C. Freeze the reagent in aliquots to avoid repetitive freeze/thawing cycles.
- Reagent stains cells in up to 10 overlaying layers of e.g. tissue sheets or cell aggregates. Cell labeling is stable for at least 24 hours.
- 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-T is a highly effective and fast live-cell dye. 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 fusion of tissue sheets in one µ–Dish $^{35mm,\ high}$ (volume 1 ml, growth area 3.5 cm^2).

Fusion of adherent tissue sheets

Note:

You also can use tissue sheets with the protocol for the fusion of tissue sheets in suspension.

1. Sonicate Fuse-It–T 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.

2. Dilute 5 µl* of the fusogenic mixture in 500 µl 1 × PBS by vortexing for 30 seconds.
   Note: Keep all components below room temperature!
3. Sonicate dilution in a standard ultrasonic bath for 5 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.

4. Replace the culture medium of the tissue sheets with the diluted fusogenic mixture.
5. Incubate for 10 minutes* at 37°C.
6. Replace the fusogenic mixture with fresh culture medium to stop fusion.
7. After fusion, the tissue sheets are immediately available for further experiments.

*For optimization of the fusion process see page 4.

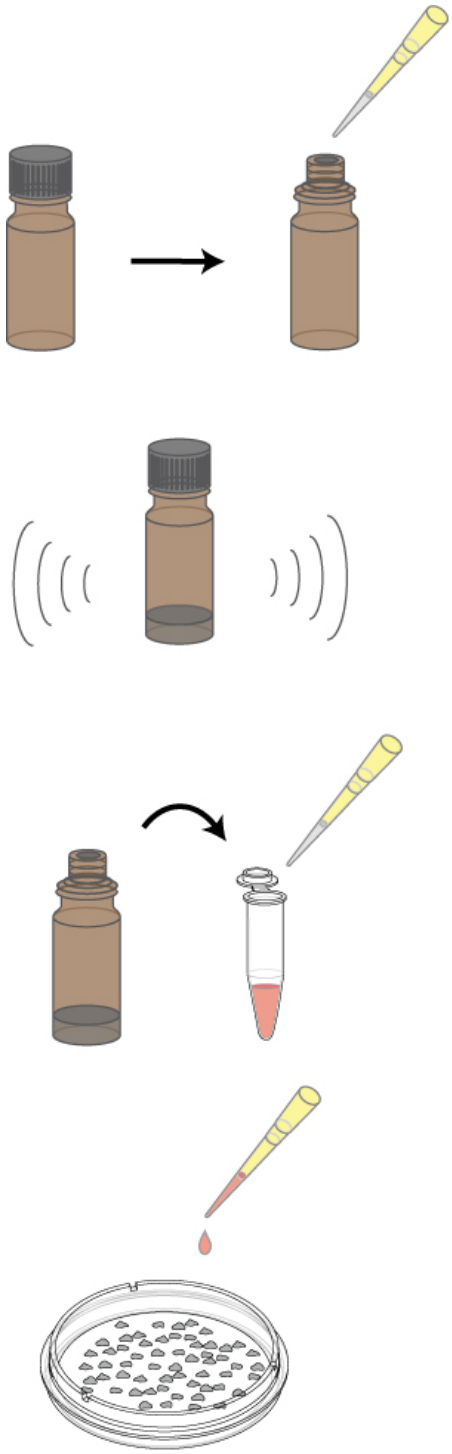

Figure 1: Schematic overview of the Fuse-It-T system with adherent tissue sheets.
Find more information on www.ibidi.com.

Fusion of tissue sheets in suspension

1. Sonicate Fuse-It–T 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.

2. Dilute 5 µl* of the fusogenic mixture in 500 µl 1 × PBS by vortexing for 30 seconds.
   Note: Keep all components below room temperature!
3. Sonicate Fuse-It–T in a standard ultrasonic bath for 5 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.

4. Centrifuge the tissue sheets and discard the supernatant.
5. Resuspend them in the diluted fusogenic mixture.
6. Incubate tissue sheets in suspension for 10 minutes at 37°C.
7. Stop fusion by adding 1 ml 1 × PBS.
8. Wash tissue sheets after centrifugation with 1 × PBS, once, or resuspend them directly in fresh culture medium.
9. After fusion, the tissue sheets are immediately available for further experiments.

*For optimization of the fusion process see page 4.

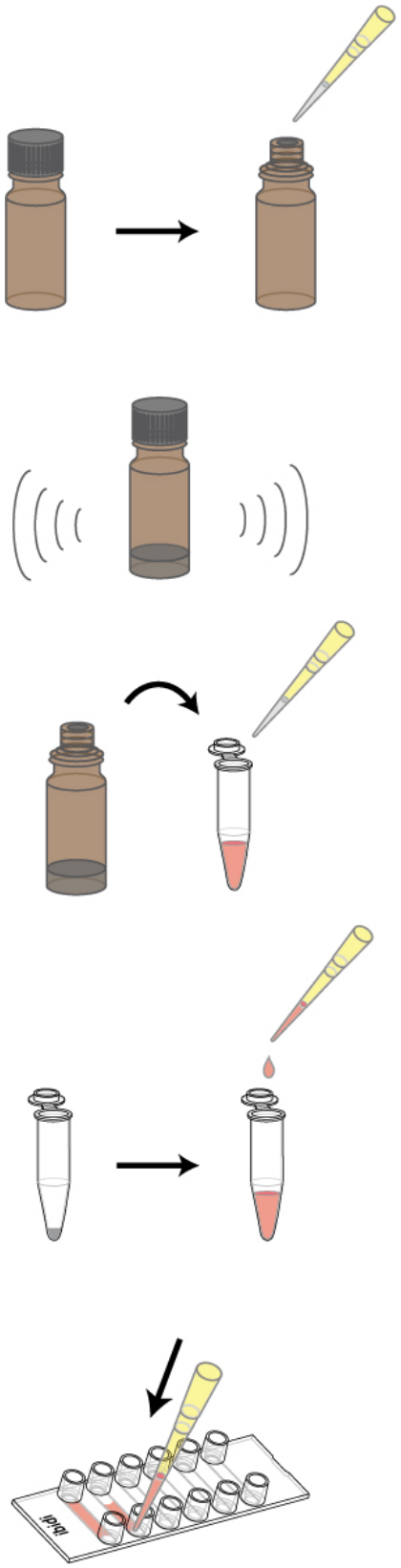

Figure 2: Schematic overview of the Fuse-It-T system with tissue sheets in suspension.
Find more information on www.ibidi.com.

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 10 – 30 minutes for the fusogenic mixture on tissue sheets.
- Gentle motion during incubation improves fusion efficiency.
- Instead of using 1 × PBS, 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-T required for successful fusion may vary slightly depending on the cell type.
- Depending on cell type, tissue sheets might re-adhere slightly slower after fusion. If necessary, use the protocol for fusion of adherent tissue sheets.
- In case very thick tissue cell layers are analyzed, free accessibility of Fuse-It-T to all sides of the tissue sheets improves staining efficiency and depth.

Fuse-It-T

Ordering Number	Labeling	Fluorescence ($Ex._{max}$ / $Em._{max}$)	Amount	
60260	Fuse-It-T	549/565 nm	100	µl
60261	Fuse-It-T	549/565 nm	400	µl

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.