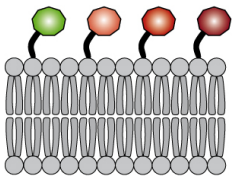

Membrane fusion is a novel and highly superior method to incorporate various molecules and particles into mammalian cells, and a strong strategy for functional studies and therapeutic approaches. Specific liposomal carriers are able to attach and instantly fuse with plasma membranes in a physicochemical-driven 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-Color is a proprietary formulation reagent made for stable, biocompatible plasma membrane labeling – within minutes – of a wide range of mammalian cells.

This reagent can be added to adherent cells, as well as to cells in suspension, independent of medium conditions. After fusion, cells can immediately be used for further analysis and labeling is typically stable for 24 hours depending on the cell type.

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	$Ex._{max}$ / $Em._{max}$
Fuse-It^{green}	484/501 nm
Fuse-It^{red}	549/565 nm
Fuse-It^{dred}	644/665 nm
Fuse-It^{IR}	750/780 nm

Additional Material Required

An ultrasonication bath is necessary with a power of 100 to 800 W and a frequency of 30 to 40 kHz. An ultrasonication probe is also possible, but then the probe has to be cooled thoroughly during a sonification in pulsed mode.

Important Guidelines

- Fuse-It-Color is solubilized in a 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, use only glass vials.
- 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-Color 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

Cell Preparation of Adherent Cells

One day before the experiment, seed the cells so as to reach ~ 90% confluence at the time of fusion.

Fusion of Adherent Cells

To fuse adherent cells with the liposomes of Fuse-It-Color, please follow these steps.

Tip:

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

1. Vortex Fuse-It–Color for 2 minutes.
2. Sonicate Fuse-It–Color 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.

3. Dilute the fusogenic mixture 1:100 in 1× PBS by vortexing for 30 seconds.*
   Note: Keep all components below room temperature!
4. 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.

5. Replace the culture medium of the cells with the diluted fusogenic mixture.
6. Incubate for 2 minutes at 37°C.*
   Note: Reaching 37°C is very important!
7. Replace the fusogenic mixture with fresh culture medium to stop fusion.
8. **Optionally** wash the cells with 1× PBS or fresh culture medium.
9. After fusion, the cells are immediately available for further experiments.

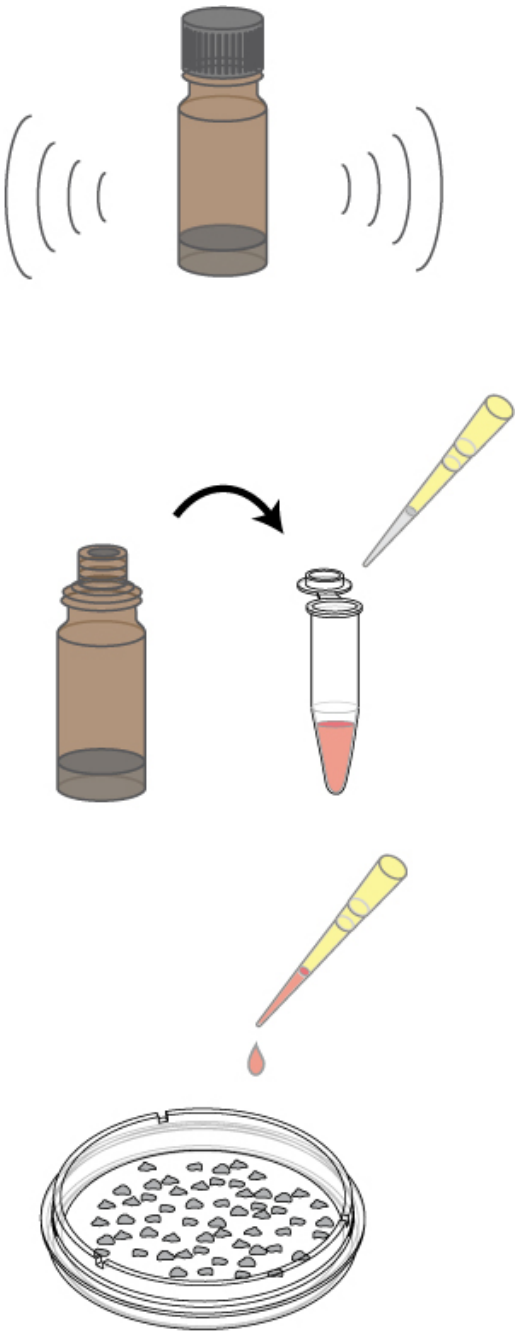

Figure 1: Schematic overview of the Fuse-It-Color system with adherent cells.

*For optimization of the fusion process see page 4.

Cell Preparation of Suspension Cells

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

Fusion of Suspension Cells

To fuse cells in suspension with the liposomes of Fuse-It-Color, please follow these steps.

1. Vortex Fuse-It–Color for 2 minutes.
2. Sonicate Fuse-It–Color 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.

3. Dilute the fusogenic mixture 1:100 in 1× PBS by vortexing for 30 seconds.*
   Note: Keep all components below room temperature!
4. 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.

5. Centrifuge the cells and discard the supernatant.
6. Resuspend the cell pellet in the diluted fusogenic mixture.
7. Incubate cells in suspension for 1-3 minutes at 37°C.*
   Note: Reaching 37°C is very important!
8. Stop fusion by adding 2 volumes of 1× PBS.
9. Centrifuge cells at an elevated speed (600 to 800 × g).
   Note: At normal speed, the cells largely remain in supernatant due to liposomal fusion.
10. Wash cells after centrifugation with 1× PBS, once, or resuspend them directly in fresh culture medium.
11. After fusion, the cells are immediately available for further experiments.

*For optimization of the fusion process see page 4.

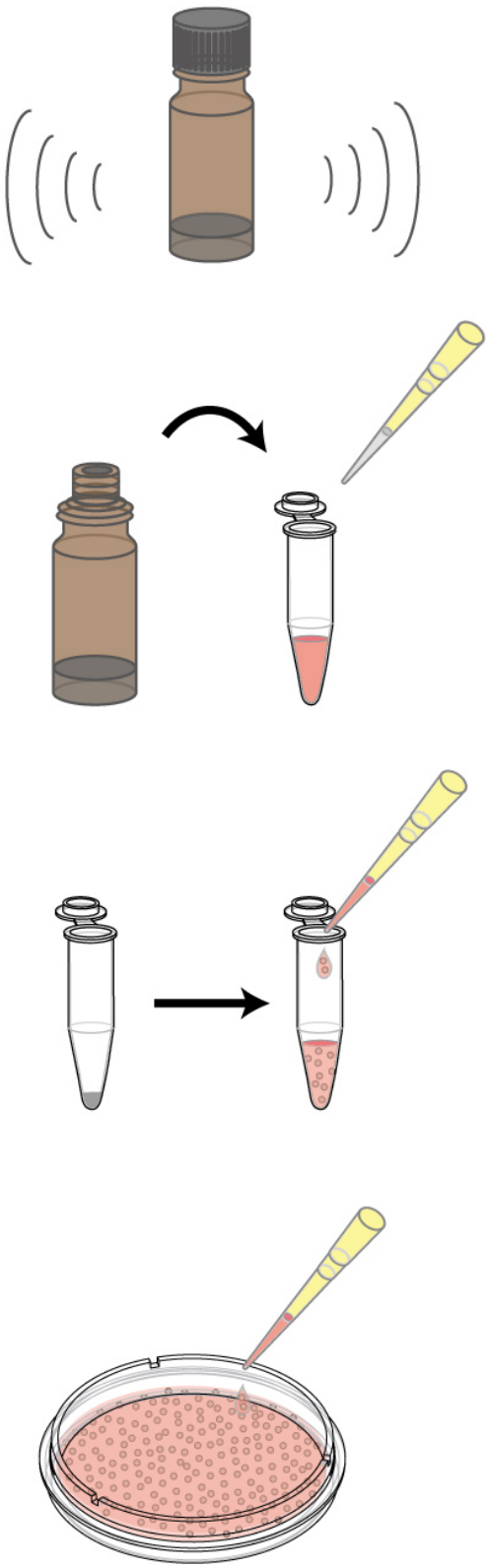

Figure 2: Schematic overview of the Fuse-It-Color system with suspension cells.

Optimization of the Fusion Process

Results may vary slightly between cell types. You can identify the optimal condition for each cell type by adjusting the incubation time, the dilution, the dilution medium, and the cell confluence.

- If necessary, the incubation time and the volume of the fusogenic mixture can be further adjusted.
  - Vary the dilution of the fusogenic mixture between 1:50 and 1:100.
  - Vary the incubation time between 1-10 minutes for the fusogenic mixture on cells.
- 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.
- High confluencies may be helpful, but not mandatory.
- Gentle motion during incubation improves fusion efficiency.
- If cell detachment is observed during the fusion process, the incubation time should be reduced.
- The amount of Fuse-It-Color required for successful fusion may vary slightly depending on the cell type and passage number.
- Depending on cell type, cells might re-adhere slightly slower after fusion. If necessary, use the protocol for fusion of adherent cells.

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

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.