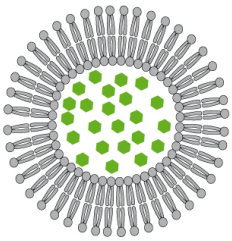

Membrane fusion is a novel and highly superior method to incorporate various molecules and particles into mammalian cells. Specific liposomal carriers are able to attach and instantly fuse with plasma membranes in a physicochemical-driven manner. Therefore, membrane fusion is a strong strategy for functional studies and therapeutic approaches.
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-P is a proprietary formulation reagent used for transferring proteins and peptides – within minutes – into the cytoplasm of a wide range of mammalian cells.

The liposomal carriers, loaded with your peptide or protein, can be added to adherent cells, as well as to cells in suspension. Plus, transferred proteins are instantly active inside the cells and, after fusion, cells can immediately be used for further analysis.

## Specifications

The logistical handling and fluorescence properties are listed in the following table:

| | |
|---|---|
| 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

| |
|---|
| Peptide-/ Protein solution (0.01–0.1 mg/ml) |
| HEPES buffer (20 mM) |

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

- If possible prepare your peptides/proteins in BSA-free buffers of low osmolarity (e.g., 20 mM HEPES) and a pH that is between 7.0 and 8.0.
- The recommended initial concentration of peptide/protein is 0.01 - 0.1 mg/ml. Depending on protein size and charge this concentration might vary and therefore the optimal concentration should be optimized.
- Following additives will obstruct the transport of peptides/proteins: Glycerol (>5 %) and BSA.
- For first time fusions, we recommend different incubation times and protein/peptide concentrations in Fuse-It-P for incubation with cells, in order to determine the best fusion efficiencies.
- After resuspension in a protein/peptide containing buffer, Fuse-It-P should be used within 24 hours to achieve the best results.
- Fusion efficiency can be visualized directly after fusion by fluorescence microscopy. Make sure to use appropriate IR filter sets and an IR sensitive camera.
- After fusion, the IR fluorescence signal can also be used for cell sorting by flow cytometry.
- 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-P is a highly effective and fast peptide and protein transfer system. Incubation times of as little as one minute might already be sufficient for receiving high transfer rates. Therefore, prolonged incubation times will not improve fusion efficiencies, might harm the cells instead.

## Protocol

The protocols are designed for the transport of one peptide/protein using Fuse-It-P for 25 µl, or Fuse-It-P for 100 µl, or Fuse-It-P for 400 µl.
Blue (marked with a •) denotes values for Fuse-It-P for 25 µl (60221);
Red (marked with a ⋆) denotes values for Fuse-It-P for 100 µl (60220 and 60223);
Cyan (marked with a △) denotes values for Fuse-It-P for 400 µl (60222).

### 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 transfer peptides/proteins into adherent cells with Fuse-It-P, please follow these steps:

**Tip:**

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

1. Add • 10 µl, or ⋆ 40 µl, or △ 100 µl of the peptide/protein solution to a Fuse-It-P vial and mix thoroughly by vortexing until complete resuspension.
2. Sonicate the mixture in a standard ultrasonic bath for 5-10 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. Fill up the mixture with 20 mM HEPES buffer (pH 7.4) to make a total volume of • 25 µl, or ⋆ 100 µl, or △ 400 µl, then vortex for 30 seconds.
4. Dilute the fusogenic mixture 1:50 with 1× PBS. Vortex for 30 seconds.*
   **Note:** Keep all components below room temperature!
5. Replace the culture medium of the cells with the diluted mixture.
6. Incubate for 5 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.

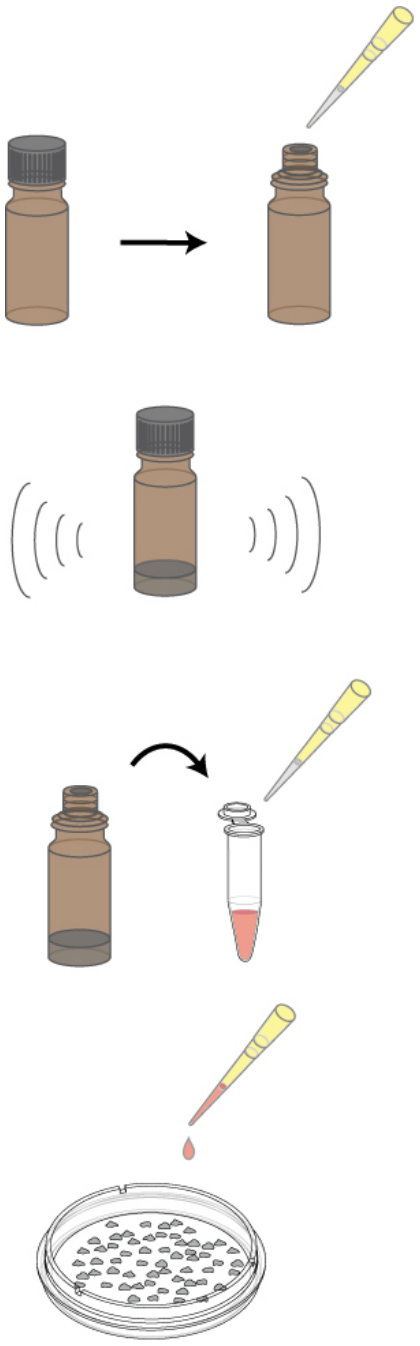

Figure 1: Schematic overview of the Fuse-It-P protocol with adherent cells.

*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 transfer peptides/proteins into cells in suspension with Fuse-It-P, please follow these steps:

1. Add • 10 µl, or ⋆ 40 µl, or △ 100 µl of the peptide/protein solution to a Fuse-It-P vial and mix thoroughly by vortexing until complete resuspension.
2. Sonicate the mixture in a standard ultrasonic bath for 5 – 10 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. Fill up the mixture with 20 mM of HEPES buffer (pH 7.4) to make a total volume of • 25 µl, or ⋆ 100 µl, or △ 400 µl, then vortex for 30 seconds.
4. Dilute the mixture 1:50 with 1× PBS. Vortex for 30 seconds.*
   **Note:** Keep all components below room temperature!
5. Centrifuge the cells as usual and discard the supernatant.
6. Resuspend the cell pellet in the diluted 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.

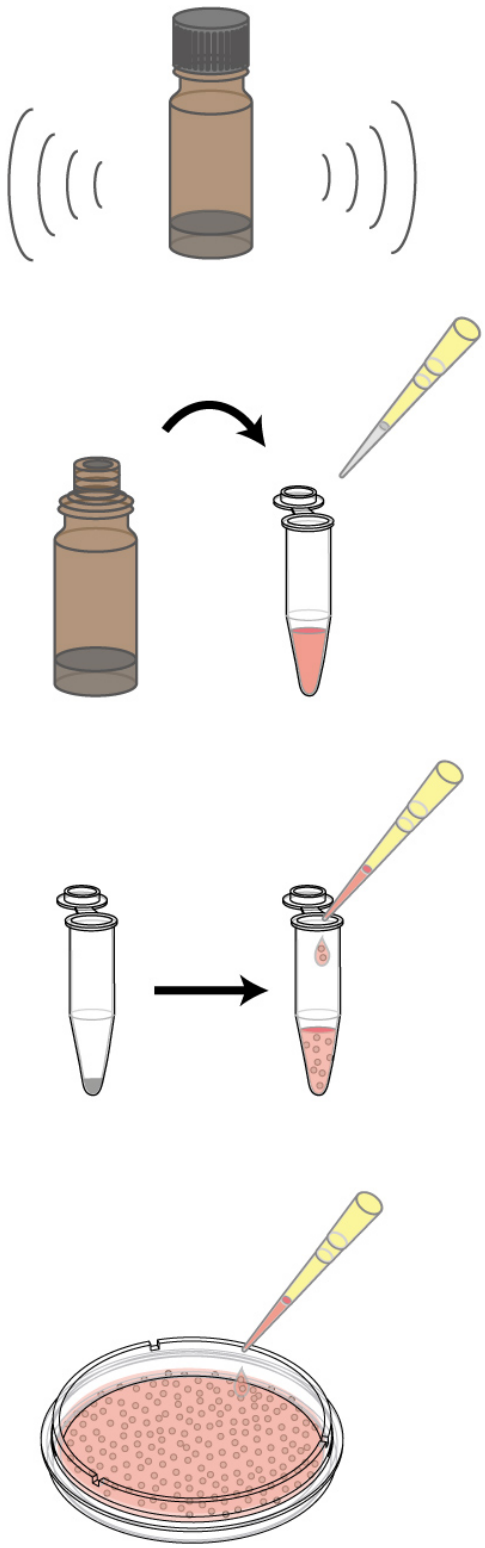

Figure 2: Schematic overview of the Fuse-It-P protocol with suspension cells.

*For optimization of the fusion process see page 4.

## Optimization of the Peptide/Protein Transfer with Fuse-It-P

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

- If necessary, the protein/peptide concentration, the incubation time and the volume of the fusogenic mixture can be further adjusted.
  - Vary the initial protein/peptide concentration between 0.01 and 0.1 mg/ml.
  - Vary the dilution of the fusogenic mixture between 1:20 and 1:50 in 1× PBS.
  - Vary the incubation time between 1-15 minutes for the fusogenic mixture on cells.
- The complete removal of the lyophilized reagent from the glass vial surface by adding the protein/peptide solution is highly relevant for successful protein/peptide delivery. For resuspension of the lyophilized Fuse-It-P in the inner vial (• Fuse-It-P for 25 µl), slightly open the screw cap before vortexing so the inner vial can move freely. Additional resuspension of the solution by pipeting up and down will result in a homogenous solution.
- 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.
- High confluences may be helpful, but are 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-P 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-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.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.