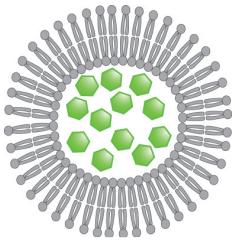


Membrane fusion is a highly efficient method for transfecting various molecules and particles into mammalian cells, even into sensitive and primary cells. The Fuse-It reagents are cargo-specific liposomal carriers that attach and rapidly fuse with plasma membranes in a physiochemical-driven manner, thus releasing their cargo into the cytoplasm of the target cell. Membrane fusion is an effective tool for functional studies, which works independently 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 into the cytoplasm of a wide range of eukaryotic cells—within minutes.
The liposomal carriers, loaded with your peptide or protein, can be added to adherent cells, as well as to cells in suspension. In addition, transferred proteins are instantly active inside the cells and, after fusion, cells can immediately be used for further analysis.

Specifications

Specifications of Fuse-It-P	
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)

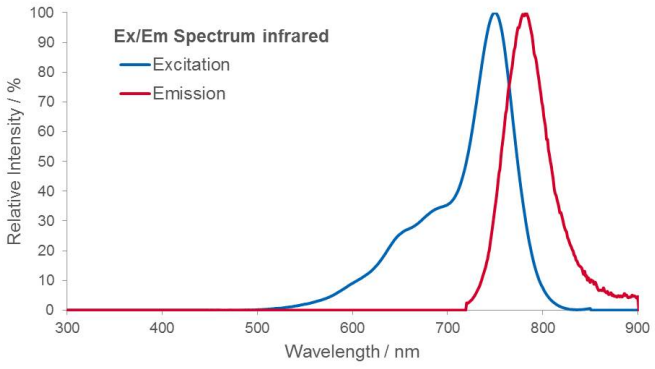


Figure 1: Spectrum of Fuse-It-P fusion control dye.

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

- Peptide-/Protein solution (0.01–0.1 mg/ml)
- HEPES buffer (pH 7.0–9.0; 20 mM)
- 1× PBS (pH 7.0–9.0; 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

- The recommended initial concentration of the peptide/protein solution is 0.01–0.1 mg/ml.
- 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 9.0. The following additives will obstruct the transport of peptides/proteins: Glycerol (>5 %) and BSA.
- After resuspension in a peptide/protein containing buffer, Fuse-It-P should not be stored. Any added molecules might affect the stability of Fuse-It-P. Also, the overall stability of the molecule of interest may vary.

- For the first use of Fuse-It-P, 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 transfer of peptides/proteins into cells in an open format using:

• Fuse-It-P for 25 µl	60221
⋆ Fuse-It-P for 100 µl	60220 and 60223
△ Fuse-It-P for 400 µl	60222

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

Note:

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

pH Adjustment for Protein Charge

The pH of the buffers has to be adjusted between 7.0 and 9.0 to reach a slightly negative protein charge. Ideally the pH is at 7.4, to assure best physiological conditions for the cells.

Note:

Because a protein can have different charges at different pH, the pH of the buffers has to be adjusted in these protocols. The appropriate pH values for different protein charges are listed in table 1.

Protein charges can be calculated by open source programs, e.g. http://protcalc.sourceforge.net/ or http://pepcalc.com/.

Table 1: pH adjustment for different protein charges.

Protein charge at pH 7.4	adjust buffer to pH
negative	7.0–7.4
neutral	7.4–8.0
positive	8.0–9.0

Cell Preparation of 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-P, please follow these steps:

1. Check if the charge of your peptide/protein is negative. If not, adjust the pH of the used buffers (HEPES and PBS) between 7.0 and 9.0 according to table 1.
2. Add • 10 µl, or ⋆ 40 µl, or △ 100 µl of the peptide/protein solution to one Fuse-It-P vial and vortex until the solution is homogeneous. If necessary, mix by pipetting.
3. Sonicate the mixture in a standard ultrasonic bath for 5–10 minutes at room temperature, or lower.[1]
4. Fill up the mixture with 20 mM HEPES buffer (adjusted pH) to make a total volume of • 25 µl, or ⋆ 100 µl, or △ 400 µl, and mix thoroughly.
5. Dilute 20 µl of the fusogenic mixture 1:50 in 1× PBS (adjusted pH) 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. **Optionally** wash the cells with 1× PBS before fusion.
9. Incubate for 5 minutes at 37°C.
   Note: Reaching 37°C is very important!
10. Replace the fusogenic mixture with fresh culture medium to stop the fusion.
11. **Optionally** wash the cells with 1× PBS or fresh culture medium.
12. After fusion, the cells are available for further experiments.

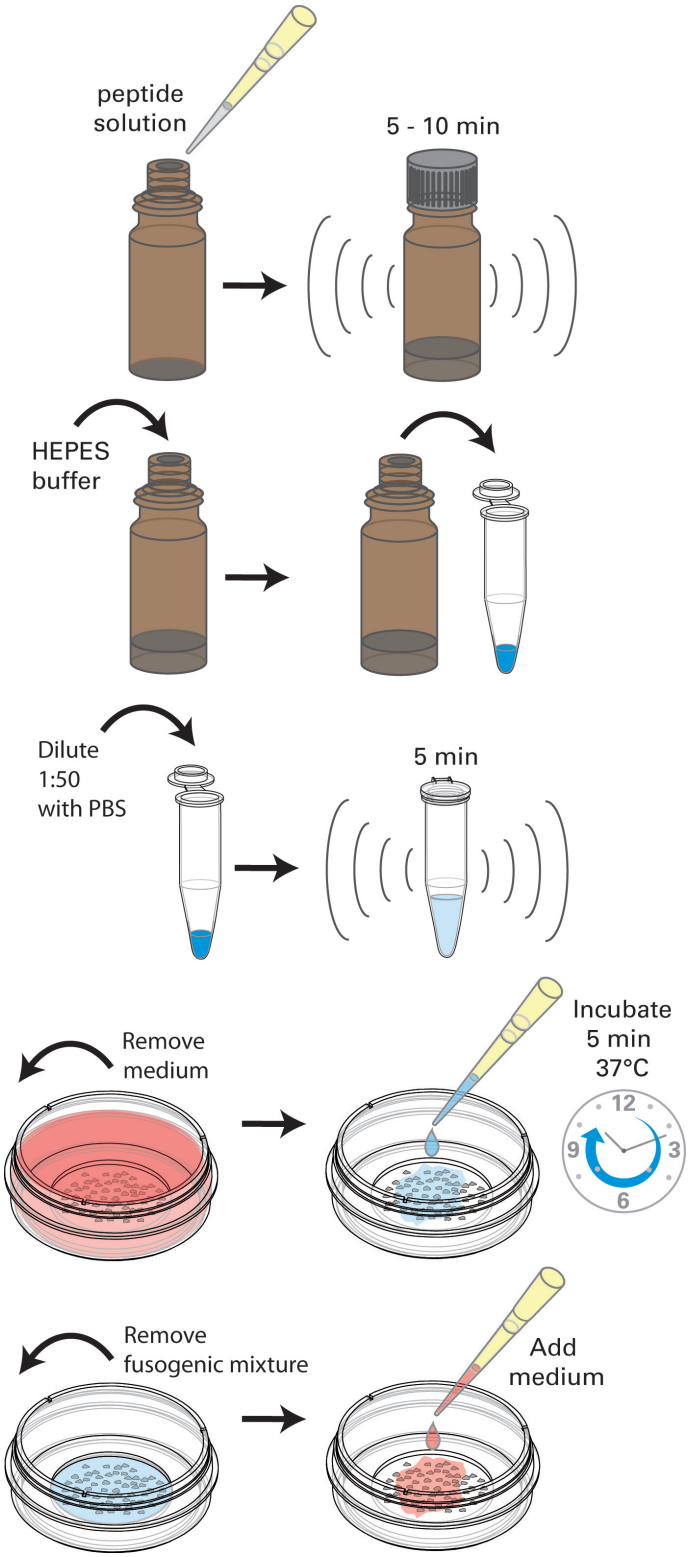


Figure 2: Schematic overview of the Fuse-It-P protocol with adherent cells.

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

Cell Preparation of 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-P, please follow these steps:

1. Check if the charge of your peptide/protein is negative. If not, adjust the pH of the used buffers (HEPES and PBS) between 7.0 and 9.0 according to table 1.
2. Add • 10 µl, or ⋆ 40 µl, or △ 100 µl of the peptide/protein solution to one Fuse-It-P vial and vortex until the solution is homogeneous. If necessary, mix by pipetting.
3. Sonicate the mixture in a standard ultrasonic bath for 5–10 minutes at room temperature, or lower.[1]
4. Fill up the mixture with 20 mM HEPES buffer (adjusted pH) to make a total volume of • 25 µl, or ⋆ 100 µl, or △ 400 µl, and mix thoroughly.
5. Dilute 20 µl of the fusogenic mixture 1:50 in 1× PBS (adjusted pH) 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.

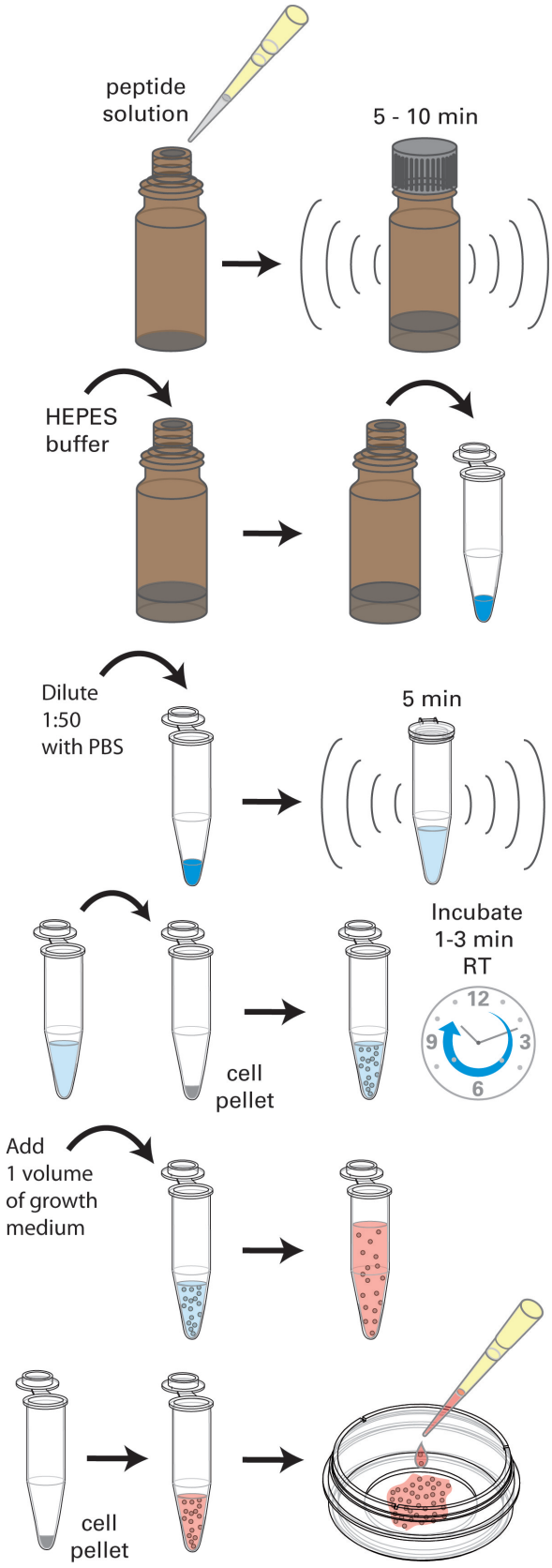


Figure 3: Schematic overview of the Fuse-It-P protocol with suspension cells.

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 initial protein/peptide concentration between 0.01 and 0.1 mg/ml. Depending on protein size and charge this concentration might vary.
  - 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 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-P for 25 µl, slightly open the screw cap before vortexing so the inner vial can move freely. If necessary, mix by pipetting.
  - Increase the concentration of ions by adding 1–5 µl of 1× PBS.
- The amount of Fuse-It-P 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 transfer peptides/proteins into 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	20.0 µl	1000 µl
µ–Slide 8 Well (ibidi)	1.0 cm^2	6.0 µl	300 µl
µ–Plate 96 Well (ibidi)	0.55 cm^2	4.0 µl	200 µl
96–well	0.3 cm^2	2.8 µl	140 µl
48–well	0.75 cm^2	6.0 µl	300 µl
24–well	1.9 cm^2	12.0 µl	600 µl
12–well	3.8 cm^2	20.0 µl	1000 µl
6–well	9.5 cm^2	40.0 µl	2000 µl

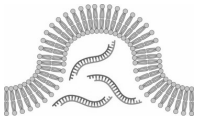
*Growth area varies depending on the manufacturer.

Suspension Cells

To transfer peptides/proteins into cells in suspension, the volumes and amounts increase linearly with the cell number.

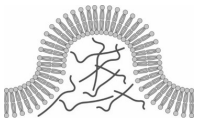
Ordering Information

Fuse-It-siRNA: Transfer of siRNA



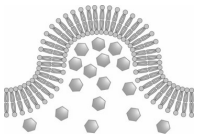
Cat. No.	Description	Amount
60510	**Fuse-It-siRNA, infrared fluorescent:** ready to use, 6 mM	2 × 150 µl
60511	**Fuse-It-siRNA, infrared fluorescent:** ready to use, 6 mM	2 × 300 µl

Fuse-It-mRNA: Transfer of mRNA



Cat. No.	Description		Amount
60505	**Fuse-It-mRNA**	**infrared fluorescent:** ready to use, 6 mM	2 × 150 µl
60506	**Fuse-It-mRNA**	**infrared fluorescent:** ready to use, 6 mM	2 × 300 µl

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.beniag.com. For questions and suggestions please contact us by e-mail info@beniag.com. All products are developed and produced in Germany.

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