

## mRNA Transfer into iPSC-derived Cardiomyocytes Using Fuse-It-mRNA in the ibidi $\mu$ -Slide VI<sup>0.4</sup>

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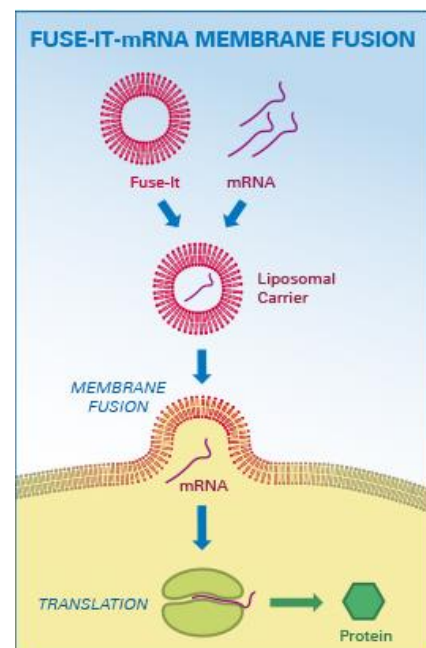
### 1. General Information

This protocol describes the optimized technique for handling Fuse-It-mRNA in ibidi channel slides, specifically the  $\mu$ -Slide VI<sup>0.4</sup>. Compared to an open well format, the advantages of the channel slide are: 1) excellent phase contrast over the entire observation field 2) very homogeneous cell distribution and 3) much lower amount of mRNA required. As an example, a fusion experiment of iPSC-derived cardiomyocytes with Fuse-It-mRNA and an eGFP-mRNA is described here.

### 2. Background

The Fuse-It liposomal carrier which contains the mRNA simply fuses immediately upon contact with the cell membrane and releases the mRNA directly into the cytoplasm (see Figure 1). The mRNA translation starts immediately without the interfering processes of endocytosis, lysosomal degradation, or mitosis. Unlike classical lipoplex-based delivery methods, cells do not internalize the mRNA by endocytosis.

The eGFP-mRNA which is used in this protocol will express an enhanced version of the green fluorescent protein originally isolated from the jellyfish, *Aequorea victoria*. eGFP is a commonly used direct-detection reporter in mammalian cell culture, yielding bright green fluorescence with an emission peak at 509 nm. The eGFP-mRNA mimics a fully processed and mature mRNA. It is capped and polyadenylated, thus optimized for mammalian systems.



**Figure 1: Simplified illustration of the membrane fusion mechanism of Fuse-It-mRNA.**

### 3. Material and Equipment

The following equipment and instruments are required for this protocol:

- Ultrasonic bath (power 50 W, frequency 35 kHz)
- Fluorescence microscope equipped with an appropriate filter set for eGFP (fluorescence properties:  $E_{x_{max}}$  483 nm /  $E_{m_{max}}$  509 nm) and a stage top incubator (37°C, 5% CO<sub>2</sub>)

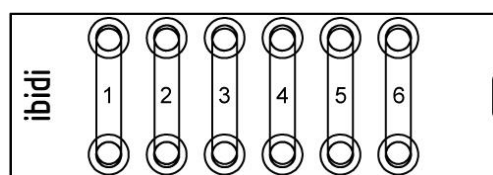
**Table 1: Material and reagents needed for the fusion of iPSC-derived cardiomyocytes.**

Name	Company	Cat. No.
Cardiomyocytes, iPSC-derived	Various	Various
Growth Medium (with FBS)	Various	Various
$\mu$ -Slide VI <sup>0.4</sup> ibiTreat	ibidi GmbH	80606
Fuse-It-mRNA	ibidi GmbH	60500
eGFP-mRNA	TriLink BioTechnologies, Inc.	L-6301
1x PBS	Sigma-Aldrich	D8537
Microreaction tube (RNase-free)	Various	Various

### 4. Experimental Procedure

The best optical confluence for fusion experiments with Fuse-It-mRNA is 70-90% or higher. The cell seeding concentration, the cultivation time, and the surface coating can all be adjusted to optimize the confluence. If necessary, perform a pre-experiment with a cell concentration series or a suitable protein coating. A detailed protocol for different proteins can be found in [Application Note 08](#) on [www.ibidi.com](http://www.ibidi.com).

In this Application Note, the effect of the amount of mRNA and the incubation time was tested. See Table 2 for experimental details. It is recommended to perform three biological replicates per condition which is why the following protocol is adapted for three  $\mu$ -Slide VI<sup>0.4</sup>. Preparing all solutions as triplicates increases the volumes to a level where pre-dilutions are not necessary. If preparing one  $\mu$ -Slide VI<sup>0.4</sup> only, pre-dilutions will become necessary. In this case, please follow the protocol for pre-dilution in the [Instructions of Fuse-It-mRNA](#).



**Figure 2: Schematic drawing of  $\mu$ -Slide VI<sup>0.4</sup> with six channels per slide.**

**Table 2: Experimental layout for the fusion of cardiomyocytes in the  $\mu$ -Slide VI<sup>0.4</sup>. All values are given per channel (30  $\mu$ l).**

Channel	1	2	3	4	5	6
Fusogenic Solution per channel	0.25 $\mu$ l					
Amount eGFP-mRNA per channel	50 ng		100 ng		200 ng	
Incubation at 37°C	4 min	8 min	4 min	8 min	4 min	8 min

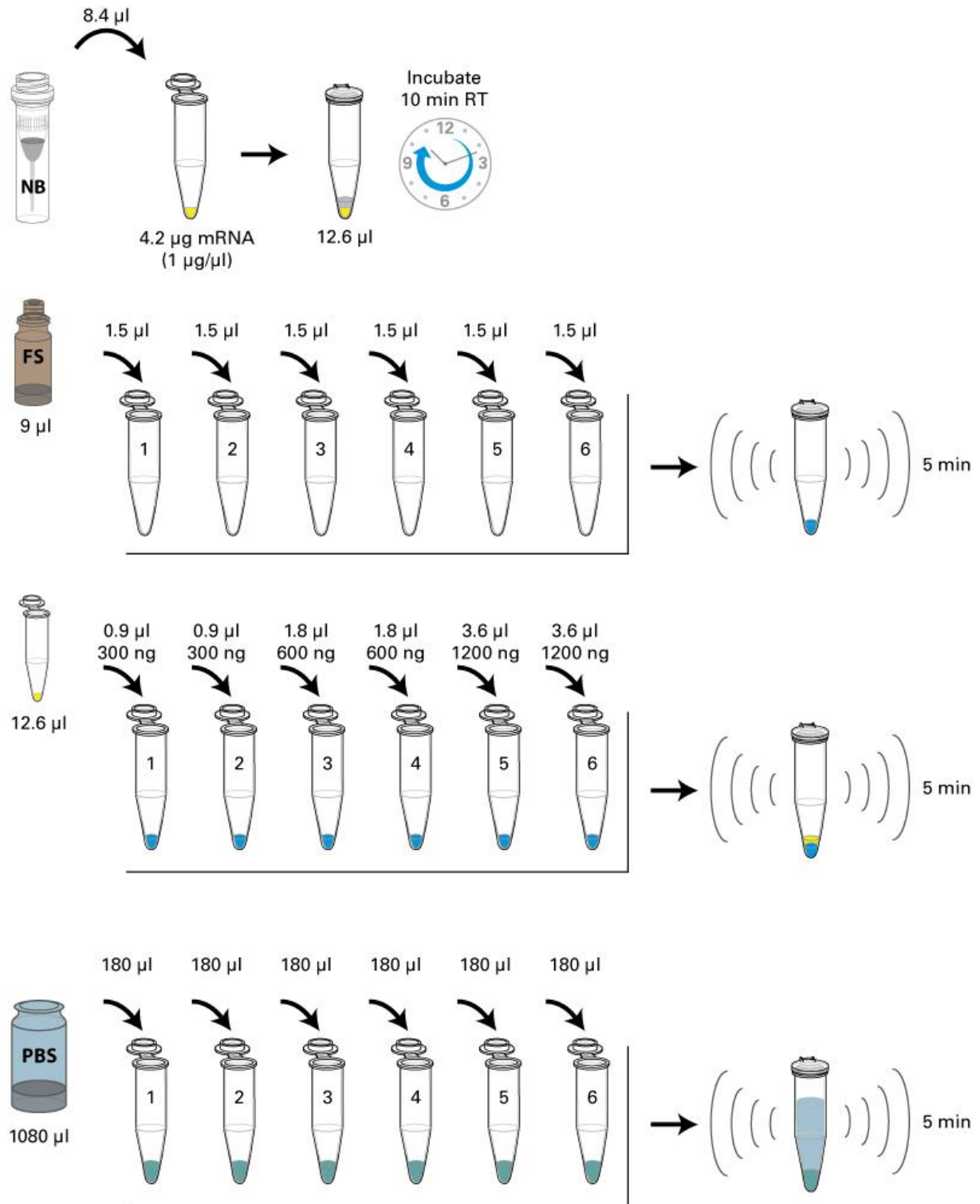
### a) Seeding Cells

1. Seed 30  $\mu\text{l}$  cell suspension of the cells per channel with a concentration of  $2.33 \times 10^6$  cells/ml (=70.000 cells per channel).
2. Incubate the cells for 2 hours at 37°C under standard cell culture conditions.
3. Add 120  $\mu\text{l}$  of growth medium per channel to fill the Luer reservoirs.
4. Incubate the cells for 24 hours at 37°C under standard cell culture conditions to reach >70% confluence at the time of fusion.

### b) Preparation of mRNA Filled Fusogenic Liposomes

See Figure 3 for a detailed pipetting scheme.

1. Cool down the ultrasonic bath with an ice pack.
2. Thaw the eGFP-mRNA on ice.
3. Thaw the Neutralization Buffer (NB) and the Fusogenic Solution (FS) at room temperature or lower.
4. Preparation of mRNA:
  - i) Mix 4.2  $\mu\text{g}$  of mRNA (= 4.2  $\mu\text{l}$  of 1  $\mu\text{g}/\mu\text{l}$  stock) and 8.4  $\mu\text{l}$  of NB thoroughly.
  - ii) Incubate for 10 min at room temperature.
5. Preparation of FS:
  - i) Vortex the FS until the solution is homogeneous. If necessary, mix by pipetting.
  - ii) Transfer 1.5  $\mu\text{l}$  of the FS into each of six microreaction tubes.
  - iii) Sonicate all six tubes in the ultrasonic bath for 5 min at room temperature or lower. If necessary, add another ice pack.
6. Mixing mRNA and FS:
  - i) Add 0.9  $\mu\text{l}$  / 1.8  $\mu\text{l}$  / 3.6  $\mu\text{l}$  of the neutralized mRNA (mRNA + NB) to the FS.
  - ii) Sonicate all six tubes in the ultrasonic bath for 5 minutes at room temperature or lower.
7. Dilution with PBS:
  - i) Dilute the fusogenic mixture with 180  $\mu\text{l}$  1x PBS per microreaction tube and mix thoroughly.
  - ii) Sonicate all six dilutions in the ultrasonic bath for 5 minutes at room temperature or lower.



**Figure 3: Full pipetting scheme for three technical replicates and 60 µl volume necessary per channel. Please note that for each channel 60 µl are prepared and used for pipetting, while only 30 µl will remain in the channel.**

### c) Fusion Protocol

1. Channels 1, 3 and 5 (incubation time 4 min)
  - i) Remove all medium from the Luer reservoirs. Do not empty the channel. Leave the channel filled with medium.
  - ii) Add 60 µl of pre-warmed 1x PBS to one side of the channel and remove 60 µl from the other side.
  - iii) Per channel, add 60 µl of the final fusogenic mixture to one side of the channel and remove 60 µl from the other side.
  - iv) Incubate at 37°C for 4 minutes.
  - v) Add 60 µl of medium to one side of the channel and remove 60 µl from the other side to stop the fusion.
  - vi) Add 120 µl of medium to the channel to fill the reservoirs.
2. Channels 2, 4 and 6 (incubation time 8 min)
  - i) Repeat all steps mentioned above but incubate at 37°C for 8 minutes.
3. Incubate the cells for 24 hours at 37°C under standard cell culture conditions.
4. Image cells by phase contrast and fluorescence microscopy.

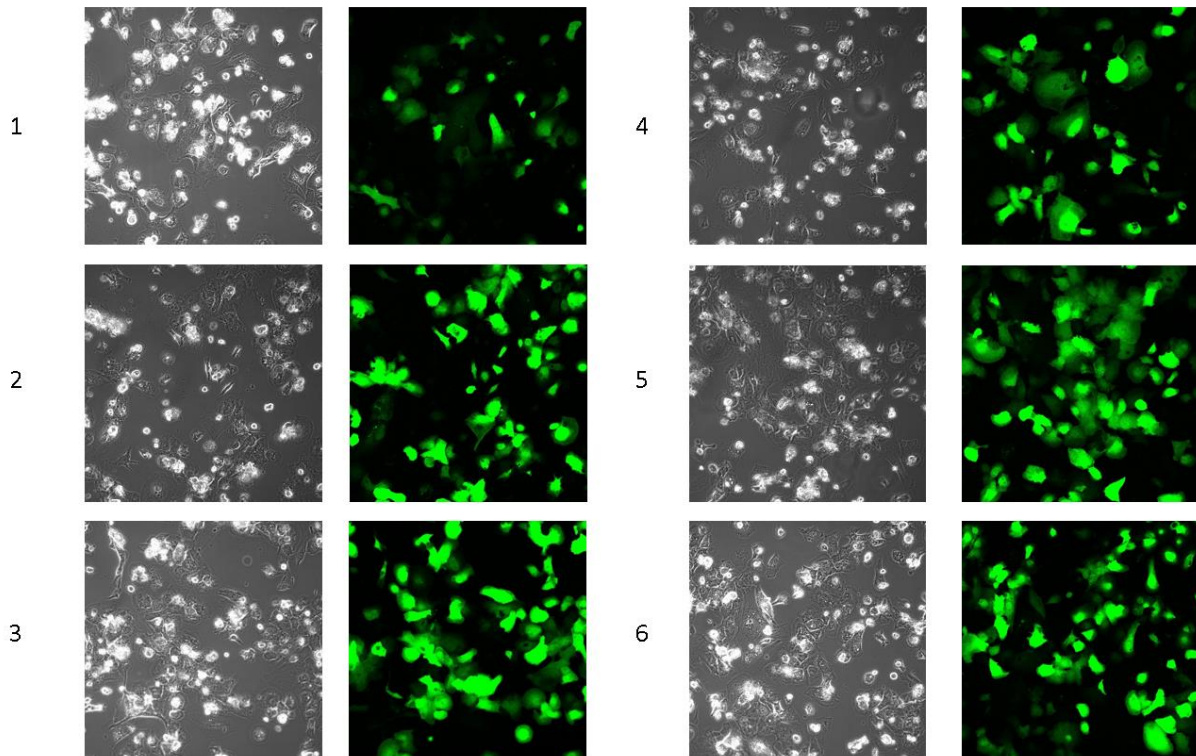
**Note:** Do not remove the liquid completely from the channels. At all times 30 µl of liquid must remain in each channel.

### d) Expression Analysis

For each sample the eGFP-mRNA expression-efficiency was determined by fluorescence microscopy after 24 hours with representative images. In addition to fluorescence imaging, phase contrast pictures were taken.

## 5. Results

Cardiomyocytes can be analyzed two hours after fusion-dependent eGFP-mRNA transfer. Due to full biocompatibility, no cell recovery time is necessary. The cells are unaffected by Fuse-It-mRNA liposomal fusion. The fusion efficiency can also be checked immediately after incubation of the cells with the fusogenic liposomes by fluorescence microscopy by utilizing the infrared fluorescence of Fuse-It-mRNA. In this protocol at least 90% of all cells are fused. The simultaneous mRNA transfer efficiency is between 70% and 90%. The eGFP protein concentration increases with time and reaches its maximum after approximately 12-24 hours. This level remains stable for up to 10 days.



**Figure 4: Resulting microscopic images 24 hours after fusion.**

In conclusion, the fusion of cardiomyocytes in the  $\mu$ -Slide VI <sup>0.4</sup> works well. The best results are achieved when using 100 ng of mRNA per channel with a 4 min incubation time (Channel 4).

Please keep in mind that incubation time and mRNA concentration may slightly vary for other cell types.

## 6. Optimization

The expression intensity and the homogeneity can be adjusted further by fusion time adaptation, mRNA concentration, or by repeated fusion.

For repeated fusion, the incubation time is split into two halves. Repeated fusion is done by following this short protocol:

1. Prepare 60  $\mu$ l final fusogenic mixture per channel.
2. For the first fusion, per channel, add 30  $\mu$ l of the final fusogenic mixture to the left side of the channel and remove 30  $\mu$ l from the right side.
3. Incubate at 37°C for 50% of the desired incubation time.
4. For the second fusion, per channel, add 30  $\mu$ l of the final fusogenic mixture to the right side of the channel and remove 30  $\mu$ l from the left side.
5. Incubate at 37°C for the remaining incubation time.
6. Add 60  $\mu$ l of medium to one side of the channel and remove 60  $\mu$ l from the other side to stop the fusion.
7. Add 120  $\mu$ l of medium to the channel to fill the reservoirs.