ScreenFect®mRNA

Transfection Reagent

Product Information & Instruction Manual

Cat. No. of ScreenFect®mRNA

S-5001

S-5001-2

S-5001-3

InCella

Contents

1. Characteristics of ScreenFect®mRNA

ScreenFect®mRNA is a specialized reagent for the successful delivery of mRNA into mammalian cells.

ScreenFect®mRNA is serum compatible and free of animal derived components. There is no need for medium change after transfection. ScreenFect® Reagents have relatively low cytotoxicity, allowing easy One-Step[1] cell transfection of recently detached cells. This One-Step procedure reduces the duration of your experiment by one day. Once diluted, ScreenFect®mRNA can be used for a period of up to four days. Always mix the dilution directly before use.

2. General Information

2.1 Handling of ScreenFect® Transfection Reagents

ScreenFect® Reagents should be mixed by vortexing before each use. Do not aliquot and store ScreenFect® Reagents in containers other than the one it is delivered in as contact of undiluted liposomal reagents with plastic surfaces may reduce performance. Dilute by pipetting ScreenFect® Reagents directly into the supplied Dilution Buffer, avoiding contact with the side of tubes, and use pipette action to wash out traces remaining in the pipette tip.

2.2 Storage Conditions

ScreenFect® Reagents should be stored at 2-8°C. Do not leave for extended periods at room temperature and do not freeze. After storage of several months without using the reagent slight precipitation may occur. This has no influence on the performance of ScreenFect® Reagents. If kept refrigerated at 2-8°C the shelflife of ScreenFect® Reagents is guaranteed for at least 12 months.

[1] One-Step transfection: transfection method in which cell plating and transfection is performed in one single step (*see also 3.2.1*)

2.3 Shipping

Shipment is done at ambient temperature to reduce environmental waste and cost. We see no significant loss of activity with storage at room temperature for periods up to two weeks.

2.4 Technology

At Incella, we combine our expertise in chemistry and biology to create an interdisciplinary research and development environment that encompasses chemical design, synthesis, liposomal reagent preparation, cell-based screening assays as well as identification and final optimization of novel transfection reagents. With respect to the chemical synthesis, our proprietary thiol-yne based combinatorial click chemistry method allows parallel, high-throughput synthesis of hundreds of novel lipid-like molecules. The method is cost effective, highly efficient and allows for the synthesis of lipids of diverse chemical structures.

3. Basic Considerations for Successful Transfection

3.1 Cells

Cells used for transfection should be in exponential growth phase and have a relatively even density over the entire surface area on which they are plated. We recommend splitting cells when they reach 80 % - 90 % confluence to avoid contact inhibition of cell proliferation. Cells should be mycoplasma free.

3.2 Transfection Methods

Cells can be transfected using the two different methods described below.

3.2.1 One-Step Transfection (Combined Plating & Transfection)

For One-Step transfection (also referred to as **Reverse Cell Transfection**), freshly detached cells in suspension are added to the transfection complexes. The transfection process is thus initiated before cell attachment takes place.

Important facts and benefits of One-Step transfection:

- **Time efficient** procedure (combined plating and transfection)
- **highly recommended for ScreenFect® Reagents**
- Due to the low cytotoxicity of our reagents, reverse cell transfection **does not harm the cells but…**
- **significantly increases transfection efficiencies for most cell lines** tested.

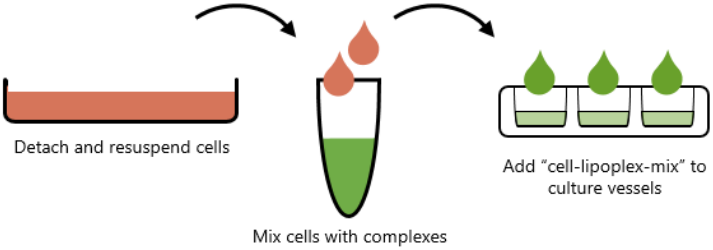


Figure 1: One-Step transfection method

3.2.2 Two-Step Transfection

In the Two-Step or Forward Transfection Method, the cells are plated 24 hours before transfection. The next day, complexes of transfection reagent and nucleic acid are added to the already adherent cells. For more information see *chapter 5. Troubleshooting*.

3.3 Nucleic Acid

For best transfection results, ensure a high quality of your mRNA stock. The amount of mRNA required per transfection can vary depending on the construct. Therefore, it is important to determine the appropriate amount of mRNA per transfection through optimization experiments (*see section 4.3.2*).
Negatively charged nucleic acid combines with the cationic lipids of ScreenFect® Reagents to form transfection complexes. Transfection complex formation is affected by the mRNA-to-Liposome Ratio, expressed as µg mRNA to µl ScreenFect® Reagent (undiluted). A 1:2 ratio represents a mixture which contains, for example, 0.1 µg mRNA and 0.2 µl of ScreenFect® Reagent.

3.4 ScreenFect® Reagent Dilution and Complex Formation

Transfection complex formation is a critical step for optimal transfection results. The nucleic acid and the transfection reagent must be evenly mixed in Dilution Buffer, both as their separate dilutions as well as when subsequently combined for transfection complex formation. If previously diluted ScreenFect® Reagents are to be used again (up to 4 days storage possible) mix by vortexing immediately before addition to mRNA.

For optimal mixing at onset of transfection complex formation, equal volumes of diluted mRNA and ScreenFect® Reagent dilution are combined using fast pipette action to rapidly form a homogeneous mixture. Strong vortexing is not recommended during complex formation due to the strong shear forces that may disrupt the complex formartion process. For larger scale transfections (e.g. transfection in culture dishes) "splitting" larger volumes into smaller aliquots for the complex formation step is recommended. Ensure at least 20 min of transfection complex formation time for optimal results.

3.5 Serum and Antibiotics

Serum does not affect the performance of ScreenFect® Reagents. Although there is no clear evidence for a reduced transfection efficiency using antibiotics, we recommend avoiding Penicillin and Streptomycin during transfection.

4. ScreenFect®mRNA Transfection Protocols

The following pages contain protocols for ScreenFect®mRNA transfection. The reagent volumes suggested in those protocols are a guideline. Initial optimization is important. Therefore, an optimization protocol is provided at the end of this chapter. The optimization protocol helps users to determine the optimal amount of ScreenFect®mRNA reagent as well as nucleic acid for their particular transfection experiment and cell line.

We highly recommend the One-Step transfection protocol for all our products. However, for special applications or cell types, the Two-Step protocol may be preferable. Advices on how to use our reagents in Two-Step transfections are given in *section 5 Troubleshooting*.

4.1 Overview: How to use ScreenFect®mRNA

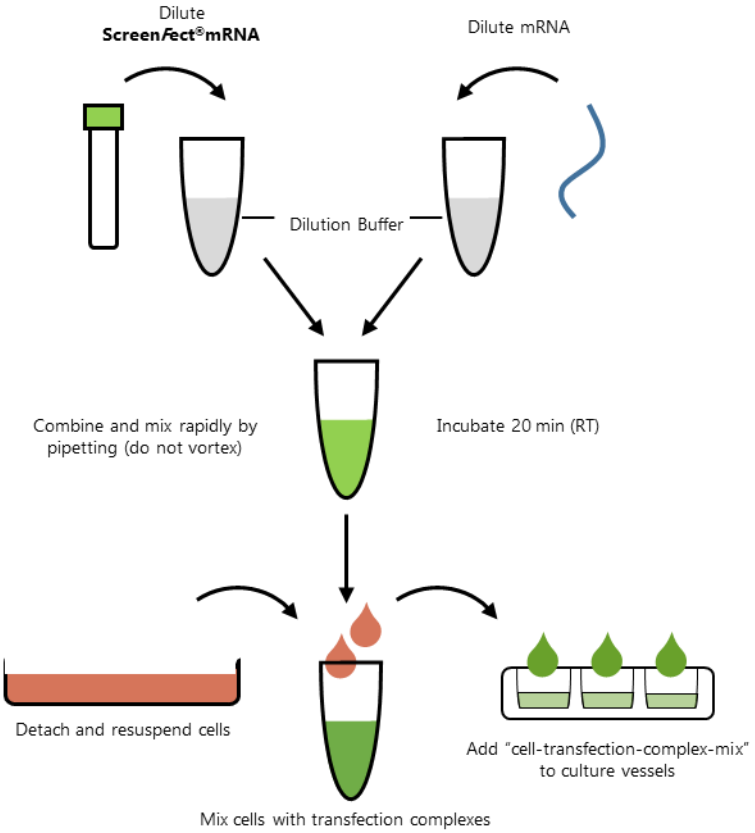


Figure 2: ScreenFect®mRNA transfection procedure at a glance

4.2 mRNA Transfection Protocols

4.2.1 Transfection of mRNA in 96-well Plate Format

Amounts shown are for one well of a 96-well plate.	
1	Dilute 0.25 µl* of ScreenFect®mRNA in Dilution Buffer to a final volume of 10 µl and mix thoroughly.
	Important: Vortex the reagent before use. Add ScreenFect®mRNA *reagent directly into supplied buffer with rapid pipette mixing or vortexing.*
2	Dilute a total of 75 ng* mRNA in Dilution Buffer to a final volume of 10 µl.
	Tip: Include a positive control for quick and easy detection of transfection (e.g. using an mRNA encoding GFP and fluorescence microscopy)
3	Combine the diluted mRNA with the ScreenFect®mRNA dilution and mix immediately using 10 rapid pipette strokes. Allow complex formation to proceed for 20 min at RT.
	Important: Do not vortex!
4	Add 80 µl freshly detached and resuspended cells to complexes and mix gently with pipette.
	Tip: The time-saving One-Step transfection method may not be suited for all cell types (see section 4.1). To transfect already adherent cells, first remove and discard medium from cells, then add 80 µl fresh culture medium to transfection complexes, mix with pipette and immediately apply to cells.
5	Transfer the cells and complexes to one well of a 96-well plate.

**Values for amounts of mRNA and reagent given in this table are recommendations. An Optimization Protocol is provided in paragraph 4.3.2. This is intended to help the user to determine the optimal amount of both* ScreenFect®mRNA *as well as mRNA for transfection of the particular cell type within one experiment.*

4.2.2 Tabular Protocol for mRNA Transfection in Selected Formats

For formats other than 96-well, scale reagents using the table below as a guideline.

Format	96-well*	24-well*	6-well*
Step 1	Dilute ScreenFect®mRNA in Dilution Buffer.		
ScreenFect®mRNA	0.25 µl	1.5 µl	5 µl
Dilution Buffer	10 µl	40 µl	120 µl
Step 2	Dilute messenger RNA in Dilution Buffer.		
Messenger RNA	75 ng	300ng	1000 ng
Dilution Buffer	10 µl	40 µl	120 µl
Step 3	Add diluted ScreenFect®mRNA to MRNA-dilution rapidly and mix with pipette.		
	20 µl	80 µl	240 µl
Step 4	After 20 min add freshly resuspended cells (or medium) to transfection complexes and transfer mixture to plate.		
Cell suspension (or medium)	80 µl	420 µl	1250 µl

**Values for amounts of mRNA and reagent given in this table are recommendations. An Optimization Protocol is provided in paragraph 4.3.2. This is intended to help the user to determine the optimal amount of both* ScreenFect®mRNA *as well as mRNA for transfection of the particular cell type within one experiment.*

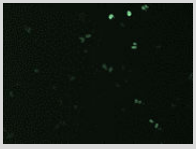
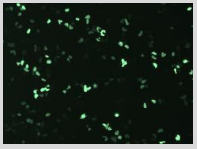
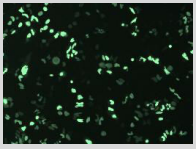
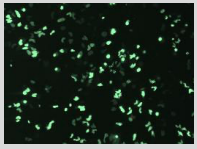
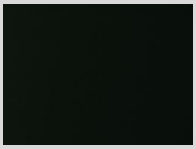
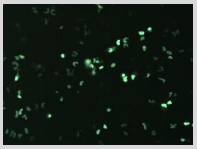
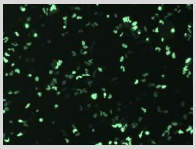
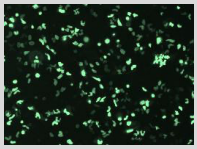
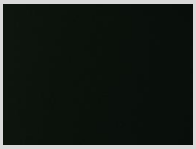
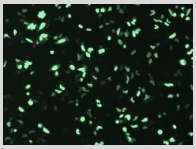
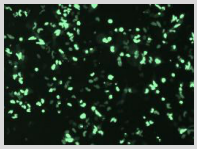
4.3 Optimization of mRNA Transfection

Optimizing transfection conditions is **highly recommended** for new users to ensure optimal transfection results. Here we provide a basic protocol for varying both the amount of ScreenFect® Reagent as well as messenger RNA in one convenient experiment.

4.3.1 Impact of Nucleic Acid (NA) to Reagent Ratio and Total Amounts of NA or Reagent on Cell Transfection Results

The following pictures show microscope images of cells 24 h after transfection. The results show that the reagent volume range of efficient cell transfection changes with changing amounts of NA used per well.

Table 1: Microscope images of cells expressing a protein accumulating in the nuclei. A 96-well format was chosen for transfection.
Note: The following pictures demonstrate how optimization and choice of conditions affect transfection results. General correlations are independent of the NA that is used. The experiment shown was performed using pDNA. The amount of pDNA was varied from 50 to 100 ng. Volumes of reagent used per well were varied between 0.1 and 0.4 µl.

reagent / pDNA	0.1 µl	0.2 µl	0.3 µl	0.4 µl
50 ng				
ratio	1:2	1:4	1:6	1:8
75 ng				
ratio	1:1.3	1:2.7	1:4	1:5.3
100 ng				
ratio	1:1	1:2	1:3	1:4

In order to find the best ratio and concentrations of mRNA and reagent for your cell line and application, you need to consider the following:

- Higher amounts of mRNA usually result in higher expression of the recombinant protein.
- Low concentrations of mRNA and reagent result in mild conditions for the cells and therefore in low cell toxicity.
- **Transfection efficiency and cell toxicity negatively correlate with each other**. It is essential to find working conditions at which these characteristics are balanced. **Be sure to choose robust conditions** *("optimal working range" in the picture below)* that are not sensitive to experimental deviations.

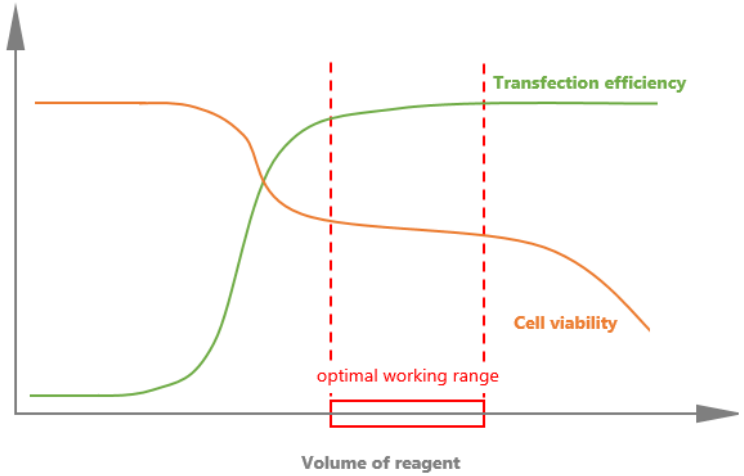


Figure 3: General correlations between reagent volumes, transfection efficiency and cell viability.

Note: Efficiency and toxicity effects are dependent on the cell type used.

4.3.2 Optimization Protocol for ScreenFect®mRNA Transfection in 96-well Plate Format

1.	Prepare 3 different messenger RNA dilutions:
1A	Take a **total** of 250 ng, 375 ng or 500 ng mRNA and dilute each amount in Dilution Buffer to a final volume of 50 µl.
	*We recommend using an mRNA encoding GFP for a first optimization, as GFP is easily monitored by fluorescence microscopy.
1B	Take 4 x 10 µl from *each* of the above 3 mRNA samples and distribute between the wells of a 96-well PCR tube plate as shown in the pipetting scheme under 3. (10 µl of diluted reagent will be added to these 12 samples later.)
	Note: The mRNA samples can alternatively be distributed between tubes of PCR-stripes.
2.	Prepare 4 different dilutions of ScreenFect®mRNA transfection reagent:
	We recommend to use PCR stripes for dilution preparation.

Sample	Corresponding final volume of ScreenFect® Reagent per 96-well:	Volume of Dilution Buffer:	Volume of ScreenFect®mRNA
2.1	0.15 µl	**197 µl**	**3 µl**
2.2	0.2 µl	**98 µl**	**2 µl**
2.3	0.25 µl	**195 µl**	**5 µl**
2.4	0.3 µl	**97 µl**	**3 µl**

	Important: add ScreenFect® *Reagents directly into supplied buffer with rapid pipette mixing or vortexing.*

3. Mix each diluted ScreenFect® sample with the different MRNA samples:

Add 10 µl of each diluted ScreenFect® Reagent sample to each of the three different amounts of mRNA in a PCR-reaction tube, mix and incubate 20 min at RT.

Take 10 µl aliquots from the 4 ScreenFect® Reagent dilutions and add to the mRNA samples containing 50 ng (samples **1-4** for plate coordinates C5-C8 as shown below). *Immediately mix with 10 rapid pipette strokes, do not vortex!* Repeat for the mRNA samples containing 75 ng (samples **5-8**) and 100 ng (samples **9-12**).

Pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12	
A													mRNA
B													amount:
C					**1**	**2**	**3**	**4**					50 ng
D					**5**	**6**	**7**	**8**					75 ng
E					**9**	**10**	**11**	**12**					100 ng
F													
G													
H													

µl of ScreenFect® Reagent per well (and *sample ID*): 0.15 *2.1* | 0.2 *2.2* | 0.25 *2.3* | 0.35 *2.4*

4. Mix transfection complexes with cells:

The **One-Step** transfection method gives best results. Add 80µl freshly dissociated cells to the complexes and then mix gently with pipette before transferring to a 96-well cell culture plate.

*For the **Two-Step** method, first remove the used medium from the 96-well cell culture plate of already adherent cells. Then add 80 µl medium to transfection complexes and transfer medium and complexes to plate with adherent cells.*

Note: View next page for information regarding the optimization of mRNA transfections in different formats.

4.3.2.1 Optimization of mRNA Transfections in Selected Formats

The following table is intended to assist the user with optimization of mRNA transfections in different formats. Only the volume ranges of reagent, dilution buffer and the ranges of nucleic acid amounts are included into the table below. For operational instructions, please refer to *section 4.3.2*.

Nucleic Acid	**mRNA**		
Format	**96-well**	**24-well**	**6-well**
Step 1	Dilute ScreenFect® Reagent in Dilution Buffer.		
ScreenFect® Reagent	0.15, 0.2, 0.25, 0.3 µl	1, 1.5, 2 µl	4, 5, 6 µl
Total volume of dilution	10 µl	40 µl	120 µl
Step 2	Dilute nucleic acid in Dilution Buffer.		
Nucleic acid	50, 75, 100 ng	300, 400 ng	1000, 1400 ng
Total volume of dilution	10 µl	40 µl	120 µl
Step 3	Add diluted ScreenFect® Reagent to the nucleic acid dilutions and mix with pipette.		
Volume of complexes	20 µl	80 µl	240 µl
Step 4	After 20 min add freshly resuspended cells (or medium in case of Two-Step) to complexes and transfer mixture to cell culture plate.		
Cell suspension	80 µl	350 µl	1250 µl

5. Troubleshooting

In case of low transfection efficiency, use the troubleshooting guide below as a basis to identify the problem.

What might be the problem in case of low transfection efficiency?

Possible causes	Suggested solutions
Poor quality of mRNA or insufficient mRNA amount	• Ensure a high quality of your mRNA and check the concentration of your mRNA stock. • The mRNA used for transfection should be free of any kind of contamination. • Optimise the concentration of mRNA according to the initial optimization protocol in *section 4.3.2*.
Insufficient complex formation	• Make sure the diluted ScreenFect® Reagent and nucleic acid solutions are, after combination, mixed immediately using rapid pipette action. Do not vortex! (*See section 3.4*) • If the volume used for complex formation is greater than about 200 µl, it may help to split the complex formation step into several tubes of reduced volumes – in order to make the initial mixing step more efficient (*see section 3.4*). • Incubate the prepared mix of mRNA and ScreenFect® Reagent for at least 20 minutes at RT.
Improper mRNA-to-reagent ratio	• Optimize the mRNA-to-ScreenFect® Reagent ratio according to the optimization protocol in *section 4.3.2*.
Incorrect storage	• ScreenFect® Reagents should be stored at 2-8°C. Do not leave for extended periods at room temperature and do not freeze. Try to avoid excessive warming-cooling if used frequently by users. Remove from 2-8°C for brief periods when needed and replace at 2-8°C.

Cells are unhealthy	• The cells used for transfection should be in exponential growth phase and have even density over surface area. Make sure your cells are free of any contamination. The use of antibiotics is recommended during passaging. Ensure the density of your cell culture does not get too high or too low during the experiment and while passaging cells – always maintain timely passaging.
Inappropriate cell density or cells are not actively dividing	• The cells used for transfection should be in exponential growth phase and have even density over the entire surface area. Ensure appropriate density of your cell culture by timely passaging. This is important for both One- and Two-Step transfection protocols. • For the Two-Step method, split your cell culture early enough before transfection (15-24 h in advance). • Excessive passaging of cells decreases the transfection performance so use cells with similar passage number between experiments to ensure reproducibility. • Use a new batch of cells.
Inhibition of complex formation	• Serum does not affect the performance of ScreenFect® Reagents, but we recommend avoiding antibiotics during transfection as well as anionic inhibitors such as EDTA or dextran sulphate.
Improper protocol	• We highly recommend the One-Step transfection method for all our reagents. Based on our experience, One-Step transfection leads to increased performance of ScreenFect® Reagents compared to Two-Step. • The time-saving One-Step transfection method may not be suited for all cell types and applications. Only if One-Step transfection does not lead to the desired results test the Two-Step method: Cells are plated into the desired format the day before so that they are adherent at time of transfection. Shortly before the addition of transfection complexes to adherent cells, we recommend removing all (for weakly adherent cells, half) of the medium from the cells. In case of 96-well plates, 80 µl of fresh medium is then mixed with the transfection complexes and this mixture is then added to the adherent cells. Take care to avoid cell detachment, especially for weakly adherent cells.

6. Packaging Sizes and Ordering Information

ScreenFect®mRNA
is provided in the following packaging sizes:

- 0.2 ml ScreenFect®mRNA + 10 ml Dilution Buffer (Cat. No.: S-5001-2)
- 1 ml ScreenFect®mRNA + 50 ml Dilution Buffer (Cat. No.: S-5001)
- 5 x 1 ml ScreenFect®mRNA + 5 x 50 ml Dilution Buffer (Cat. No.: S-5001-3)

For questions about ScreenFect® Reagents or optimization of transfection protocols or in case of any problems with our products, please feel free to contact us:

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Or visit our website for more information:

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