Product Information & Instruction Manual

ScreenFect®A
ScreenFect®A-plus

Multi-Purpose Transfection Reagents

Cat. No:

ScreenFect®A

S-3001
S-3001-2
S-3001-3

ScreenFect®A-plus

S-6001
S-6001-2
S-6001-3

Contents

1. Characteristics of ScreenFect®A & ScreenFect®A-plus

General and versatile transfection reagents:

ScreenFect®A

Reagent with especially low cytotoxicity

ScreenFect®A-plus

Optimized formulation requiring less reagent per transfection

Uses of ScreenFect®A and A-plus:

- pDNA transfection
- siRNA transfection[1]

ScreenFect®A and A-plus are 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 cell transfection of recently detached cells. This One-Step[2] procedure reduces the duration of your experiment by one day. Once diluted, ScreenFect®A and A-plus can be used for a period of up to four days. Always mix the dilution directly before use.

Important consideration: Transfection performance is always a function of efficient transfection and the reagent's gentleness to the cells. We can provide you with free samples of different ScreenFect® Reagents to help you to find the optimal reagent for your cells and applications.

[1] For siRNA transfection test our specialized reagent **ScreenFect®siRNA**

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

2. General Information

2.1 Handling of ScreenFect® Transfection Reagents

ScreenFect® Reagents should be mixed by vortexing before each use. Do not aliquot or 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 (RT) 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 twelve months.

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 ScreenFect, we combine our expertise in chemistry and biology to create an interdisciplinary research and development environment that encompasses chemical design, synthesis, liposomal reagent preparation, and cell-based screening assays as well as identification and final optimization of novel transfection reagents. With respect to the chemical synthesis, our proprietary thiolyne-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 across 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.

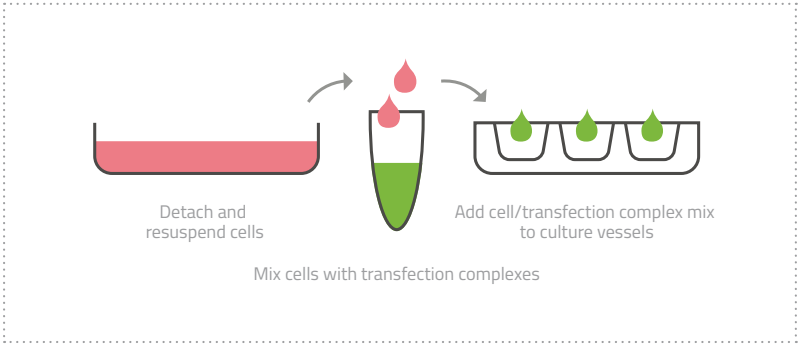


Figure 1: One-Step transfection method

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 and in fact...**
- **Significantly increases transfection efficiencies for most cell lines** tested

3.2.2 Two-Step Transfection

In the Two-Step 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 *section 5. Troubleshooting.*

3.3 Nucleic Acid

For best transfection results, the nucleic acid should be pure and endotoxin-free. An A_{260}/A_{280} ratio of 1.7–1.9 is recommended for plasmid DNA (pDNA).

The amount of any particular pDNA construct required per transfection is dependent on the gene itself, the promoter driving expression of the gene, and the plasmid backbone. Therefore, it is important to determine the appropriate amount of nucleic acid per transfection through optimization experiments *(see section 4.4.2).*

Negatively charged nucleic acid combines with the cationic lipids of ScreenFect® Reagents to form transfection complexes. Transfection complex formation is affected by the DNA-to-liposome ratio, expressed as µg DNA to µl ScreenFect® Reagent (undiluted). A 1:2 ratio represents a mixture which contains, for example, 0.1 µg pDNA and 0.2 µl of ScreenFect® Reagent.

3.4 ScreenFect® Reagent Dilution & 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 four days storage possible) mix by vortexing immediately before addition to DNA.

For optimal mixing at the onset of transfection complex formation, equal volumes of nucleic acid dilution and diluted ScreenFect® Reagent are combined using fast pipette action to rapidly form a homogeneous mixture. Vortexing is not recommended during complex formation due to the strong shear forces that may disrupt the complex formation 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 & 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, especially for siRNA transfection.

4. ScreenFect®A & ScreenFect®A-plus Transfection Protocols

The following pages contain protocols for ScreenFect®A & ScreenFect®A-plus transfection. The reagent volumes suggested in the protocols of *sections 4.2 and 4.3* 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®A or ScreenFect®A-plus 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. Advice on how to use our reagents in Two-Step transfections are given in *section 5 Troubleshooting*.

4.1 Overview: How to use ScreenFect®A & ScreenFect®A-plus

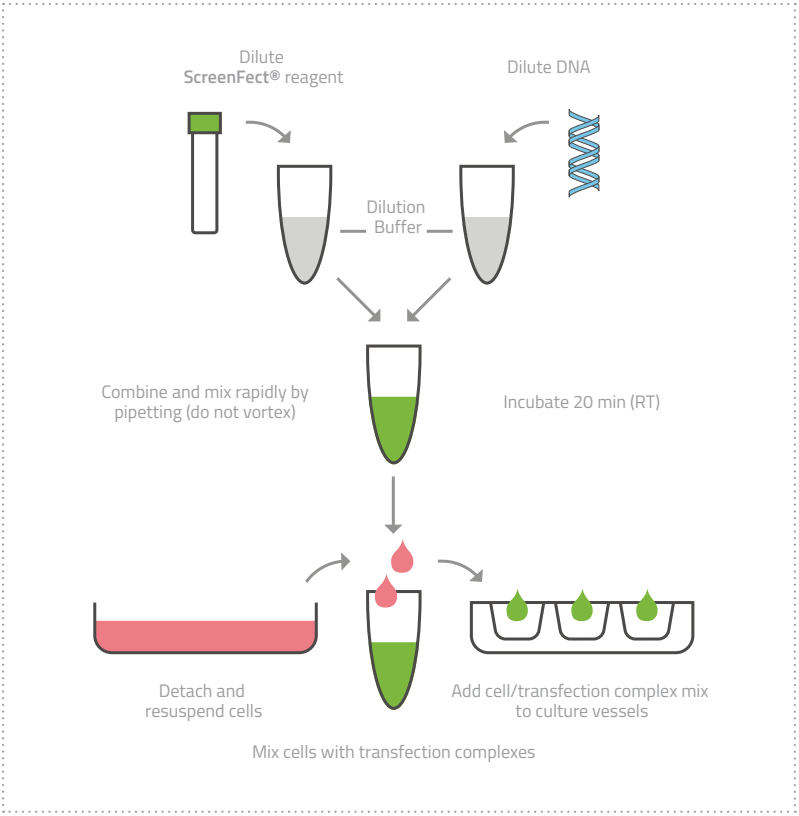


Figure 2: ScreenFect® transfection procedure at a glance

4.2 ScreenFect®A Transfection Protocols

4.2.1 Plasmid DNA Transfection Protocol

4.2.1.1 Transfection of Plasmid DNA in 96-Well Format

Amounts shown are for one well of a 96-well plate.

1. Dilute 0.35 µl* of ScreenFect®A in Dilution Buffer to a final volume of 10 µl and mix thoroughly.
   Important: *Vortex the reagent before use. Add ScreenFect®A reagent directly to supplied buffer by rapid pipette mixing or vortexing.*
2. Dilute a total of 75 ng* pDNA 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 GFP plasmid and fluorescence microscopy).*
3. Combine the diluted ScreenFect®A and DNA and mix immediately using 10 rapid pipette strokes. Leave for 20 min at RT for complex formation.
   Important: *Do not vortex!*
4. Add 80 µl of freshly detached and resuspended cells to complexes and mix gently with pipette.
   Tip: *The time-saving One-Step transfection method (see section 4.1) may not be suited for all cell types. To transfect already adherent cells, first remove and discard medium from cells, then add 80 µl of 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 pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A and nucleic acid for transfection of the specific cell type within one experiment.

4.2.1.2 Tabular Transfection Protocol for 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®A in Dilution Buffer.		
ScreenFect®A Dilution Buffer	0.35 µl 10 µl	1.7 µl 40 µl	6 µl 120 µl
Step 2	Dilute plasmid DNA in Dilution Buffer.		
Plasmid DNA Dilution Buffer	75 ng 10 µl	300 ng 40 µl	1000 ng 120 µl
Step 3	Add diluted ScreenFect®A rapidly to DNA dilution and mix with pipette.		
Volume of complexes	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

4.2.2 siRNA Transfection Protocol

Note: Although ScreenFect®A and A-plus work well for siRNA transfection, for optimal results we highly recommend the use of our specialized reagent ScreenFect®siRNA. Visit our website to order a free sample.

* Values for amounts of pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A and nucleic acid for transfection of the specific cell type within one experiment.

4.2.2.1 Transfection of siRNA in 96-Well Format

Amounts shown are for one well of a 96-well plate.

1. Dilute 0.45 µl* of ScreenFect®A to a final volume of 10 µl in Dilution Buffer and mix thoroughly.

   Important: *Vortex the reagent once per day of use. Add ScreenFect®A reagent directly to supplied buffer by rapid pipette mixing or vortexing.*

2. Dilute 1 pmol* siRNA to a final volume of 10 µl in the supplied Dilution Buffer.

3. Combine the diluted ScreenFect®A and siRNA and immediately mix using 10 rapid pipette strokes. Allow complex formation to proceed for 20 min at RT.

   Important: *Do not vortex!*

4. Add 80 µl of freshly detached and resuspended cells to complexes and mix gently with pipette.

   Tip: *The time-saving One-Step transfection method (see section 4.1) may not be suited for all cell types. To transfect adherent cells, first remove and discard medium from cells, then add 80 µl of fresh culture medium to transfection complexes, mix with pipette, and immediately apply to cells.*

5. Transfer cells and complexes to one well of a 96-well plate.

   Note: *The time required for efficient endogenous mRNA and protein depletion (24–72 h) is gene dependent and has to be determined by the end user.*

* Values for amounts of siRNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A and nucleic acid for transfection of the specific cell type within one experiment.

4.2.2.2 Tabular Protocol for siRNA Transfection in Selected Formats

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

Format	96-well*	24-well*	6-well*
Step 1	Dilute ScreenFect®A in Dilution Buffer.		
ScreenFect®A Dilution Buffer	0.45 µl 10 µl	2 µl 40 µl	4.5 µl 120 µl
Step 2	Dilute siRNA in Dilution Buffer.		
siRNA Dilution Buffer	1 pmol 10 µl	10 pmol 40 µl	30 pmol 120 µl
Step 3	Add diluted ScreenFect®A rapidly to siRNA dilution and mix with pipette.		
Volume of complexes	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 siRNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A and nucleic acid for transfection of the specific cell type within one experiment.

4.3 ScreenFect®A-plus Transfection Protocols

4.3.1 Plasmid DNA Transfection Protocol

4.3.1.1 Transfection of Plasmid DNA in 96-Well Format

Amounts shown are for one well of a 96-well plate.

1. Dilute 0.15 µl* of ScreenFect®A-plus in Dilution Buffer to a final volume of 10 µl and mix thoroughly.
   Important: *Vortex the reagent once per day of use. Add ScreenFect®A-plus reagent directly to supplied buffer by rapid pipette mixing or vortexing.*
2. Dilute a total of 75 ng* pDNA 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 GFP plasmid and fluorescence microscopy).*
3. Combine the diluted ScreenFect®A-plus and DNA and mix immediately using 10 rapid pipette strokes. Leave for 20 min at RT for complex formation.
   Important: *Do not vortex!*
4. Add 80 µl of freshly detached and resuspended cells to complexes and mix with pipette.
   Tip: *The time-saving One-Step transfection method (see section 4.1) may not be suited for all cell types. To transfect adherent cells, first remove and discard medium from cells, then add 80 µl of 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 pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A-plus and nucleic acid for transfection of the specific cell type within one experiment.

4.3.1.2 Tabular Transfection Protocol for 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®A-plus in Dilution Buffer.		
ScreenFect®A-plus Dilution Buffer	0.15 µl 10 µl	1 µl 40 µl	4 µl 120 µl
Step 2	Dilute plasmid DNA in Dilution Buffer.		
Plasmid DNA Dilution Buffer	75 ng 10 µl	300 ng 40 µl	1000 ng 120 µl
Step 3	Add diluted ScreenFect®A-plus rapidly to DNA dilution and mix with pipette.		
Volume of complexes	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

4.3.2 siRNA Transfection Protocol

Note: Although ScreenFect®A and A-plus work well for siRNA transfection, for optimal results we highly recommend the use of our specialized reagent ScreenFect®siRNA. Visit our homepage to order a free sample.

* Values for amounts of pDNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A-plus and nucleic acid for transfection of the specific cell type within one experiment.

4.3.2.1 Transfection of siRNA in 96-Well Format

Amounts shown are for one well of a 96-well plate.

1. Dilute 0.25 µl* of ScreenFect®A-plus to a final volume of 10 µl and mix thoroughly.
   Important: *Vortex the reagent once per day of use. Add ScreenFect®A-plus reagent directly to supplied buffer by rapid pipette mixing or vortexing.*

2. Dilute 1 pmol* siRNA to a final volume of 10 µl in the supplied Dilution Buffer.

3. Combine the diluted ScreenFect®A-plus and siRNA 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 of freshly detached and resuspended cells to complexes and mix gently with pipette.
   Tip: *The time-saving One-Step transfection method (see section 4.1) may not be suited for all cell types. To transfect adherent cells, first remove and discard medium from cells, then add 80 µl of fresh culture medium to transfection complexes, mix with pipette, and immediately apply to cells.*

5. Transfer the cells to one well of a 96-well plate.
   Note: *The time required for efficient endogenous mRNA and protein depletion (24–72 h) is gene dependent and has to be determined by the end user.*

* Values for amounts of siRNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A-plus and nucleic acid for transfection of the specific cell type within one experiment.

4.3.2.2 Tabular Protocol for siRNA Transfection in Selected Formats

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

Format	96-well*	24-well*	6-well*
Step 1	Dilute ScreenFect®A-plus in Dilution Buffer.		
ScreenFect®A-plus Dilution Buffer	0.25 µl 10 µl	2 µl 40 µl	3 µl 120 µl
Step 2	Dilute siRNA in Dilution Buffer.		
siRNA Dilution Buffer	1 pmol 10 µl	10 pmol 40 µl	30 pmol 120 µl
Step 3	Add diluted ScreenFect®A-plus rapidly to siRNA dilution and mix with pipette.		
Volume of complexes	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 siRNA and reagent given in this table are recommendations. An Optimization Protocol is provided in *section 4.4.2*. This is intended to help the user to determine the optimal amount of ScreenFect®A-plus and nucleic acid for transfection of the specific cell type within one experiment.

4.4 Optimization Protocol for ScreenFect®A & ScreenFect®A-plus

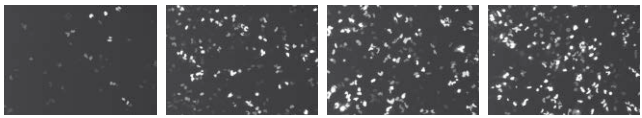
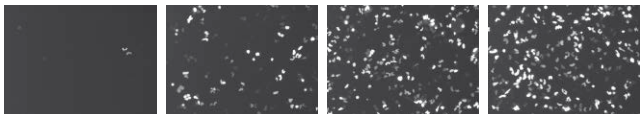
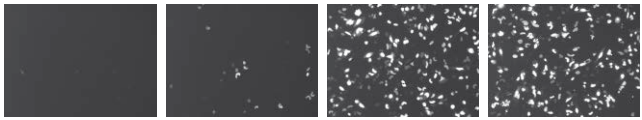
Optimizing transfection conditions is **highly recommended** for new users to ensure optimal transfection results. Here we provide a basic protocol for varying the amount of ScreenFect® Reagent and plasmid DNA in one convenient experiment.

4.4.1 Impact of pDNA-to-Reagent Ratio & Total Amounts of DNA or Reagent on Cell Transfection Results

The following pictures of transfected cells were taken with a fluorescence microscope 24 h after transfection. The Optimization Protocol *(see 4.4.2)* was strictly followed.

The results show that the reagent volume range of efficient cell transfection changes with changing amounts of pDNA used per well.

Table 1: Microscope images of cells expressing a fluorescent protein accumulating in the nuclei. A 96-well format was chosen for transfection. 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. The ratios are defined as µg of pDNA to µl of reagent.

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 pDNA and reagent for your cell line and application, you need to consider the following:

- Higher amounts of pDNA usually result in higher expression of the recombinant protein.
- Low concentrations of pDNA 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.

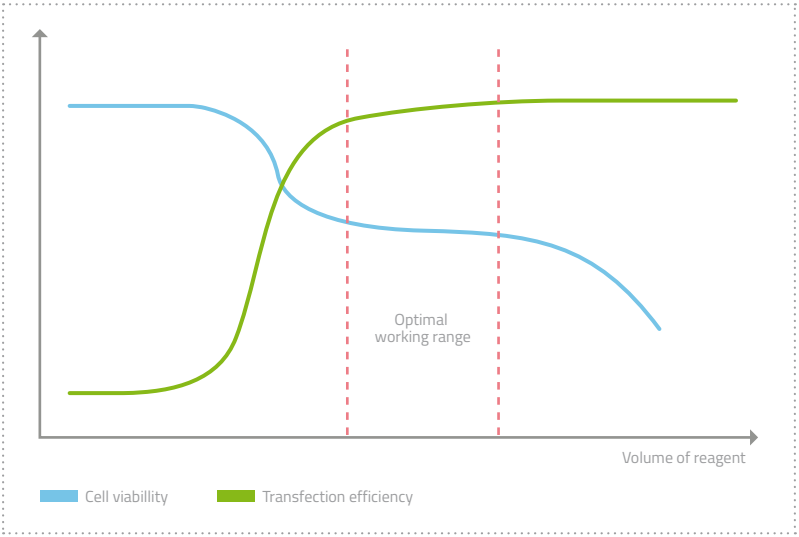


Figure 3: General correlations between reagent volumes, transfection efficiency and cell viability.

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

4.4.2 Optimizing ScreenFect®A & ScreenFect®A-plus Plasmid DNA Transfection

1 Prepare three different plasmid DNA dilutions:

A Take a total of 250 ng, 375 ng or 500 ng plasmid DNA and dilute each amount in Dilution Buffer to a final volume of 50 µl.

* We recommend using a pDNA encoding GFP for a first optimization, as GFP is easily monitored by fluorescence microscopy. Optimal transfection conditions are strongly dependent on the pDNA construct that is used. Therefore, if your target construct differs significantly (e.g. in size) from GFP pDNA, use your target pDNA and the corresponding assay for optimization.

B Take 4 x 10 µl from each of the above three DNA 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 twelve samples later.)

Note: *The DNA samples can alternatively be distributed between tubes of PCR stripes.*

2 Prepare four different dilutions of ScreenFect®A or A-plus transfection Reagent in Dilution Buffer:

We recommend the use of PCR stripes for the preparation of the reagent dilutions.

Sample ID	Corresponding final volume of ScreenFect® Reagent per 96-well:	Volume of Dilution Buffer:	Volume of ScreenFect®A or ScreenFect®A-plus:
2.1	0.1 µl	**198 µl**	**2 µl**
2.2	0.2 µl	**98 µl**	**2 µl**
2.3	0.3 µl	**97 µl**	**3 µl**
2.4	0.4 µl	**96 µl**	**4 µl**

Important: *Add ScreenFect® Reagents directly to supplied buffer and mix with rapid pipette action or vortexing.*

3

Mix each diluted ScreenFect® sample with the different DNA samples:

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

Take 10 µl aliquots from the four ScreenFect® Reagent dilutions and add to the DNA 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 DNA 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												
B												
C					**1**	**2**	**3**	**4**				
D					**5**	**6**	**7**	**8**				
E					**9**	**10**	**11**	**12**				
F												
G												
H												

pDNA amount:
50 ng
75 ng
100 ng

µl of ScreenFect® Reagent per well (and *sample ID*):				
	0.1	0.2	0.3	0.4
	2.1	2.2	2.3	2.4

4

Mix transfection complexes with cells:

The One-Step transfection method gives best results. Add 80 µl 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 containing adherent cells. Then add 80 µl of medium to transfection complexes and transfer medium and complexes to plate with adherent cells.*

Note: The Optimization Protocol (Steps 1–4) can be downloaded as a one-page PDF from our website. Information regarding the optimization of pDNA and siRNA transfections in different formats is provided on the next page.

4.4.2.1 Optimization of pDNA and siRNA Transfections in Selected Formats

The following table is intended to assist the user with optimization of both pDNA and siRNA transfections in different formats. Only the volume ranges of reagent, Dilution Buffer, and the ranges of nucleic acid amounts are included in the table below. For operational instructions, please refer to *section 4.4.2.*

Nucleic Acid	pDNA			siRNA		
Format	**96-well**	**24-well**	**6-well**	**96-well**	**24-well**	**6-well**
Step 1	Dilute ScreenFect® Reagent in Dilution Buffer.					
ScreenFect® Reagent	0.1, 0.2, 0.3, 0.4 µl	1.0, 1.5, 1.7 µl	4.0, 5.0, 6.0 µl	0.25, 0.35, 0.45 µl	0.75, 1.4, 2.0 µl	3.0, 4.5, 6.0 µl
Total volume of dilution	10 µl	40 µl	120 µl	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	1 and 2 pmol	10 and 20 pmol	30 and 60 pmol
Total volume of dilution	10 µl	40 µl	120 µl	10 µl	40 µl	120 µl
Step 3	Add diluted ScreenFect® Reagent rapidly to the nucleic acid dilutions and mix with pipette.					
Volume of complexes	20 µl	80 µl	240 µl	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	420 µl	1250 µl	80 µl	420 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 DNA or insufficient DNA amount	• Check the quality and concentration of plasmid DNA. Ideally, the ratio A_{260}/A_{280} is approximately 1.8. • The DNA used for transfection should be free of any kind of contamination. Contaminations like RNA and proteins may influence the transfection efficiency. • Optimize the concentration of DNA according to the initial Optimization Protocol in *section 4.4.2.* • Make sure the DNA which is used for transfection is endotoxin free.
Insufficient complex formation	• Make sure the diluted ScreenFect® Reagent and nucleic acid solutions are mixed immediately after combination, 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 pDNA and ScreenFect® Reagent for at least 20 minutes at RT.
Improper DNA-to-reagent ratio	• Optimize the DNA-to-ScreenFect® Reagent ratio according to the Optimization Protocol in *section 4.4.2.*

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 across the 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 across 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, as well as anionic inhibitors such as EDTA or dextran sulfate, during transfection.

Improper transfection method	• 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 test the Two-Step method if One-Step transfection does not lead to the desired results: 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.
Incorrect vector	• Make sure the promoter on the vector being used works with the cell type in your experiment. • Verify the DNA sequence.

6. Packaging Sizes & Ordering Information

ScreenFect®A

is provided in the following packaging sizes:

- 0.2 ml ScreenFect®A + 10 ml Dilution Buffer (Cat. No.: S-3001-2)
- 1 ml ScreenFect®A + 50 ml Dilution Buffer (Cat. No.: S-3001)
- 5 x 1 ml ScreenFect®A + 5 x 50 ml Dilution Buffer (Cat. No.: S-3001-3)

ScreenFect®A-plus

is provided in the following packaging sizes:

- 0.2 ml ScreenFect®A-plus + 10 ml Dilution Buffer (Cat. No.: S-6001-2)
- 1 ml ScreenFect®A-plus + 50 ml Dilution Buffer (Cat. No.: S-6001)
- 5 x 1 ml ScreenFect®A-plus + 5 x 50 ml Dilution Buffer (Cat. No.: S-6001-3)

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:

info@screenfect.com

Or visit our website for more information:

www.screenfect.com

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