Application Protocol - 4T1

ScreenFect®A-plus

ScreenFect®A-plus Transfection Reagent

Package Contents

Cat. No.	ScreenFect®A-plus	Dilution Buffer
S-6001-2 S-6001 S-6001-3	0.2 ml 1.0 ml 5 x 1.0 ml	10 ml 50 ml 5 x 50 ml

Storage Conditions

Store ScreenFect® Reagents at 4 °C. Do not freeze. For optimal long term activity, do not allow ScreenFect® Reagents to warm to room temperature each time it is used.

After several months of storage without using the reagent a slight precipitation might occur. If vortexed thoroughly, this has no influence on the performance of ScreenFect® Reagents.

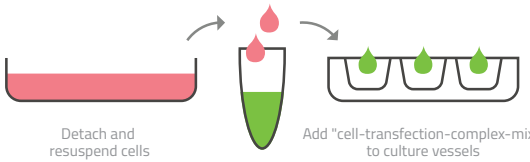
General Considerations

For optimal results, amounts of ScreenFect®A-plus and nucleic acid (NA) need to be optimized for each cell type and each NA used. An optimization protocol is provided in our product manual which can be downloaded from our homepage.

We strongly recommend the One-Step transfection method for all of our products. For transfection of adherent cells, remove the used medium and mix fresh medium with the transfection complexes. Then add the mix to the cells.

ScreenFect®A-plus is suitable for pDNA and RNA transfection (view our manual for optimized protocols). For best results in mRNA and siRNA delivery, test our specialized reagents.

ScreenFect® Protocol: One-Step Transfection



ScreenFect® Products

ScreenFect®A	Multipurpose reagent (most suitable for pDNA transfection, suitable for RNA applications) with very low cytotoxicity.
ScreenFect®A-plus	Multipurpose reagent with optimized formulation requiring less reagent per transfection.
ScreenFect®siRNA	Specialized reagent for best performance in siRNA delivery.
ScreenFect®65 + Booster	Reagent kit for protein production in HEK suspension cells.

Application Protocol - 4T1

ScreenFect®A-plus

Protocol for the transfection of **4T1** cells with pDNA

	Component	Procedure for one well (24-well-plate)	24-well
1	**Reagent Dilution**	Dilute 1.5 µl of ScreenFect®A-plus in Dilution Buffer to a final volume of 40 µl and mix thoroughly.	1.5 µl reagent 40 µl dilution
		Important: *Vortex the reagent once per day of use. Add ScreenFect®A-plus reagent directly into supplied buffer with rapid pipette mixing or vortexing.*	
2	**pDNA Dilution**	Dilute a total of 500 ng pDNA in Dilution Buffer to a final volume of 40 µl.	500 ng 40 µl dilution
		Tip: *Include a positive control for quick and easy detection of transfection (e.g. using GFP plasmid and fluorescence microscopy).*	
3	**Complex formation**	Combine the diluted ScreenFect®A-plus and DNA and mix immediately using 10 rapid pipette strokes. Leave for 20 min at room temperature for complex formation.	80 µl complexes
		Important: *Do not vortex!*	
4	**Cell preparation & transfection**	Add 420 µl freshly detached and resuspended cells to the complexes and mix with pipette.	Add 420 µl cell suspension
		Tip: *The time-saving reverse cell transfection method may not be suited for all cell types. To transfect adherent cells, first remove and discard medium from cells, then add 420 µl fresh culture medium to transfection complexes, mix with pipette and immediately apply to cells.*	
5	**Cell plating**	Transfer the cells and complexes to one well of a 24-well plate.	Transfer cells with complexes to plate

Note: This protocol is a guideline. Values are suitable for easy for transfect cell lines. This protocol does not replace optimization experiments. View our manual for instructions.
Serum does not affect the performance of ScreenFect®A-plus but we recommend avoiding antibiotics.
Cells must be mycoplasma free, in exponential growth phase and have even plating density across the entire surface area.