Quick Protocol

ScreenFect®UP-293

Protocol for adherent cell transfection with ScreenFect®UP-293

Package Contents

Cat. No.	ScreenFect®UP-293	SFA P-reagent	Dilution Buffer
S-8010-2	0.2 ml	-	20 ml
S-8010	1.0 ml	-	50 ml
S-1002-2	-	0.1 ml	-

Storage Conditions

Store ScreenFect® Reagents at 2-8°C. Do not freeze.

For optimal long term activity, do not allow ScreenFect® Reagents to warm to room temperature each time it is used.

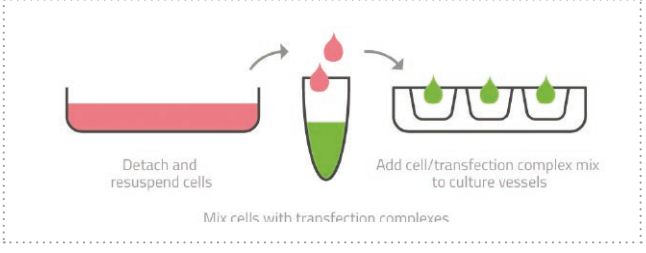
After several months of storage without using the reagent a slight precipitation might occur. If vortexed thoroughly, this has no influence on the performance of ScreenFect® Reagents.

General Considerations

For optimal results, amounts of ScreenFect®UP-293, P-reagent and nucleic acid (NA) need to be optimized for each cell line and each NA used.

An optimization protocol is provided in our product manual which can be downloaded from the ScreenFect homepage. We strongly recommend the One-Step transfection method for all of our products. For transfection of adherent cells (Two-Step protocol), remove the used medium and mix fresh medium with the transfection complexes. Then add the mix to the cells.

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®mRNA	Optimized reagent for the delivery of mRNA.

For additional information regarding ScreenFect®UP-293 and other ScreenFect® Products, visit the ScreenFect homepage (www.screenfect.com) and view our product pages and instruction manuals.

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ScreenFect®UP-293

Protocol for pDNA Transfection

	Component	Procedure for one well (96-well-plate)	96-well	24-well	6-well
1	**Reagent Dilution**	Dilute 0.3 µl of ScreenFect®UP-293 in Dilution Buffer to a final volume of 10 µl and mix thoroughly.	0.3 µl reagent 10 µl dilution	2 µl reagent 40 µl dilution	6 µl reagent 120 µl dilution
		Important: *Vortex the reagent once per day of use. Add ScreenFect®UP-293 reagent directly into supplied buffer with rapid pipette mixing or vortexing.*			
2	**pDNA Dilution**	Dilute a total of 75 ng pDNA in Dilution Buffer to a final volume of 10 µl. Add 75 nl of SFA P-reagent and proceed with step 3 within **<1min** to ensure maximum performance.	75 ng 10 µl dilution + 75 nl SFA P-reag.	300 ng 40 µl dilution + 0.3 µl SFA P-reag.	1000 ng 120 µl dilution + 1.0 µl SFA P-reag.
		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®UP-293 and DNA and mix immediately using 10 rapid pipette strokes. Leave for 20 min at room temperature for complex formation.	20 µl complexes	80 µl complexes	240 µl complexes
		Important: *Do not vortex!*			
4	**Cell preparation & transfection**	Add 80 µl freshly detached and resuspended cells to the complexes and mix with pipette.	Add 80 µl cell suspension	Add 420 µl cell suspension	Add 1250 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 80 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 96-well plate.	Transfer cells with complexes to plate	Transfer cells with complexes to plate	Transfer cells with complexes to plate

Note: This protocol is a guideline. This protocol does not replace optimization experiments. For 96-well transfection test also 0.2 and 0.25 µl of reagent. For 24-well test reagent volumes of 1-2 µl and for 6-well transfections test reagent volumes of 4-6 µl.
Serum does not affect the performance of ScreenFect®UP-293 but we recommend avoiding antibiotics.
Cells must be mycoplasma free, in exponential growth phase and have even plating density across the entire surface area.