Application Protocol

ScreenFect®A

ScreenFect®A Transfection Protocol for CRISPR/Cas9 mediated editing

Step	Component	Procedure for one well (6-well-plate)	6-well
1	Reagent Dilution	Dilute 8.4 µl of ScreenFect®A in Dilution Buffer to a final volume of 240 µl and mix thoroughly.	8.4 µl reagent 240 µl dilution
		Important: *Vortex the reagent immediately before use. Add ScreenFect® Reagent directly into supplied buffer and mix by vortexing for 2 seconds. Leave at RT for 2 min.*	
2	pDNA Dilution	Dilute a total of 2 µg of combined pDNA (e.g. donor and Cas9/guide RNA containing vectors) in Dilution Buffer to a final volume of 240 µl.	2 µg pDNA 240 µl dilution
		Important: *Mix gently by multiple pipette strokes.*	
3	Complex formation	Combine the dilutions of pDNA and ScreenFect® Reagent and mix immediately using 10 rapid pipette strokes. Leave for 20 min at RT for complex formation.	480 µl complexes
		Important: *Pipette the diluted ScreenFect® reagent into the diluted pDNA and mix with pipette. Do not vortex!*	
4	Cell preparation & transfection	Add 1520 µl freshly detached and resuspended cells to the complexes and mix gently by pipetting.	Add 1520 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 6-well plate. Start selection process 48h post-transfection (e.g. for 5 day puromycin selection)	Transfer cells with complexes to plate

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