PROTOCOL

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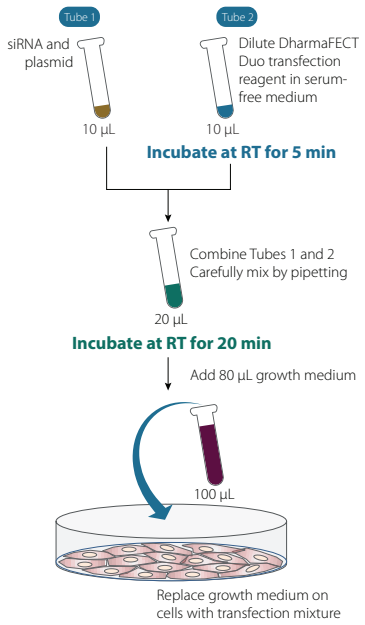
INSPIRED CELL SOLUTIONS

DharmaFECT™ Duo co-transfection protocol

The following is a protocol for co-transfecting siRNA reagents with a plasmid into cultured mammalian cells using DharmaFECT™ Duo transfection reagent (Cat #T-2010-xx). For more details please see full protocol.

This protocol is written for transfection into 96-well tissue culture plates at 100 nM final concentration of siRNA with 100 ng of plasmid (100 µL final volume).



96–well protocol			
Day 1			
Cell plating	Seed cells at a density that is appropriate for your experiment		
Day 2			
Prepare working solutions of reagents for transfection	siRNA	Dilute siRNA to a working concentration of 2 µM in 1× siRNA buffer or another appropriate RNase-free solution	
	Plasmid	Dilute plasmid to a working concentration of 20 µg/mL in 10 mM Tris-HCl pH 7.4-buffered solution or another appropriate RNase-free solution	
Combine working solutions for transfection mixture		For one well	For multiple wells
	Tube 1		
	siRNA (2 µM)	5 µL	_ µL
	Plasmid (20 µg/mL)	5 µL	_ µL
Prepare working solution of DharmaFECT Duo for transfection	Tube 2		
	DharmaFECT Duo transfection reagent	0.05–0.5 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
Combine transfection mixture	Combine Tube 1 and Tube 2 and carefully mix by pipeting		
	Incubate at room temperature for 20 minutes before next step		
	Add antibiotic-free full growth medium	80 µL	_ µL
	Total	100 µL	_ µL
Transfect cells	Replace growth medium on cells with 100 µL of transfection mixture		

If you have any questions, contact

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