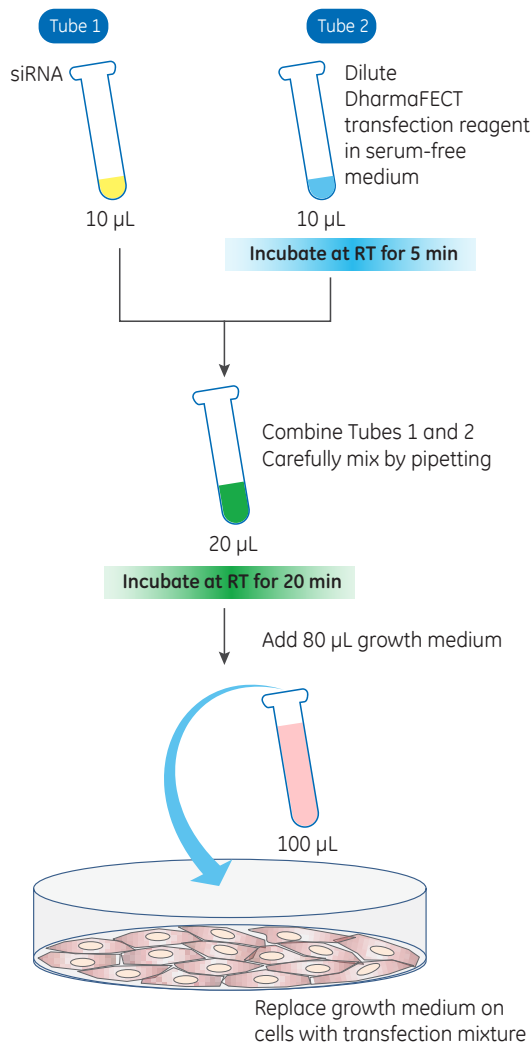


Dharmacon™ DharmaFECT™ 1–4 transfection protocol

The following is a protocol for transfecting Dharmacon™ synthetic siRNA or microRNA reagents into cultured mammalian cells using DharmaFECT™ transfection reagents: DharmaFECT 1 (Cat #T-2001-xx), DharmaFECT 2 (Cat #T-2002-xx), DharmaFECT 3 (Cat #T-2003-xx), or DharmaFECT 4 (Cat #T-2004-xx). For more details please see full [protocol](#).

This protocol is written for transfection of cells in into 96-well tissue culture plates at 25 nM final concentration of siRNA (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 5 µM in 1× siRNA buffer or another appropriate RNase-free solution	
Dilute siRNA for transfection		For one well	For multiple wells
		Tube 1	
	siRNA (5 µM)	0.5 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
Prepare working solution of DharmaFECT for transfection		Tube 2	
		DharmaFECT transfection reagent	0.05–0.5 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		

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