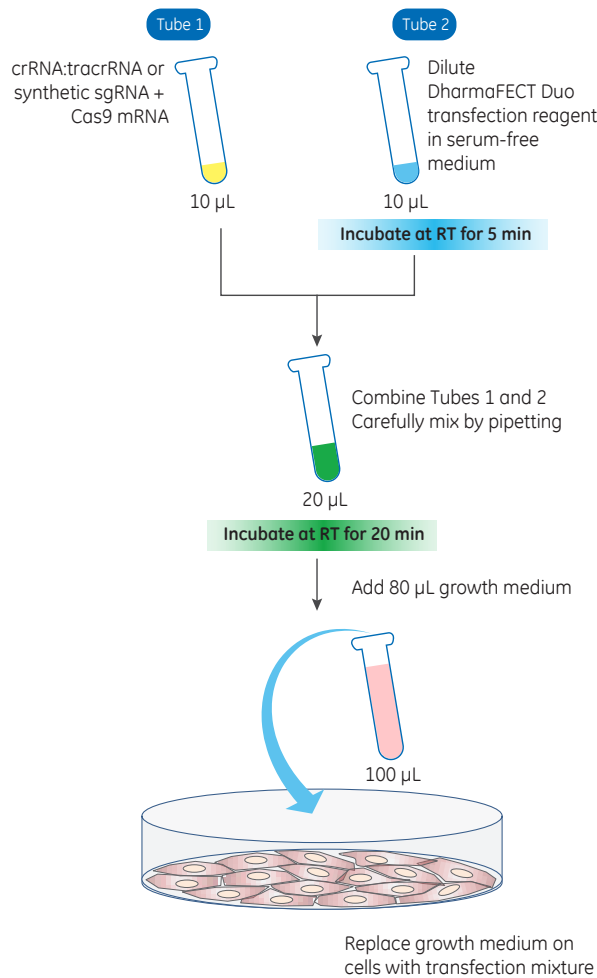


# Dharmacon™ Edit-R™ Cas9 mRNA and synthetic guide RNA transfection protocol

The following is a protocol for transfecting Dharmacon™ Edit-R™ Cas9 mRNA (Cat #CAS11195) with synthetic guide RNA into cultured mammalian cells using DharmaFECT™ Duo transfection reagent (Cat #T-2010-xx). Synthetic guide RNA can be either Edit-R synthetic tracrRNA (Cat #U-002005-xx) complexed with crRNA (predesigned or custom) or Edit-R synthetic single guide RNA (sgRNA, custom). For a more detailed protocol please see the [Technical Manual](#).

The protocol is written for transfection into 96-well tissue culture plates (100 µL final volume).



96-well protocol			
Day 1			
Cell plating	Seed cells at a density that gives 70-90% confluency on the next day		
Day 2			
Prepare working solutions of reagents for transfection	synthetic guide RNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4-7.5 or Dilute synthetic sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4-7.5	
	Cas9 mRNA	Dilute Cas9 mRNA to a working concentration of 100 ng/µL in serum-free medium	
Combine working solutions for transfection mixture		For one well	For multiple wells
	<b>Tube 1</b>		
	synthetic guide RNA	1.25 µL	_ µL
	Cas9 mRNA	2 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
Prepare working solution of DharmaFECT Duo for transfection	<b>Tube 2</b>		
	DharmaFECT Duo transfection reagent	0.1-0.8 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
<b>Incubate at room temperature for 5 minutes before next step</b>			
Combine transfection mixture	Combine Tube 1 and Tube 2 and carefully mix by pipetting		
	<b>Incubate at room temperature for 20 minutes before next step</b>		
	Add full growth medium	80 µL	_ µL
	Total	100 µL	_ µL
Transfect cells	<b>Replace growth medium on cells with 100 µL of transfection mixture</b>		

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