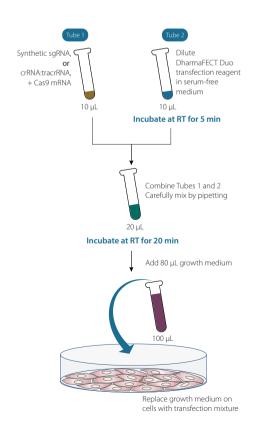
PROTOCOL



Edit-R[™] Cas9 mRNA and synthetic guide RNA transfection protocol

The following is an abbreviated protocol for transfecting Edit-R[™] Cas9 mRNA (<u>Cat #CAS11195, #CAS11859, or #CAS11860</u>) with synthetic guide RNA into cultured mammalian cells using DharmaFECT[™] Duo transfection reagent (<u>Cat #T-2010-xx</u>). Synthetic guide RNA can be either synthetic single guide RNA, or synthetic crRNA complexed with tracrRNA. Intended for use after optimization for your cell line has been completed. For full details, as well as optimization guidelines please see the <u>Technical Manual</u>.



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 sgRNA to a working concentration of 2 μ M in 10 mM Tris-HCl, pH 7.4 or Dilute and mix crRNA and tracrRNA to a working concentration of 2 μ M in 10 mM Tris-HCl, pH 7.4			
	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 mulitple wells		
	Tube 1				
	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	Tube 2				
	DharmaFECT Duo transfection reagent	0.1–0.8 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 te	mperature for 20 minutes befo	ore next step		
	Add full growth medium	80 µL	_ µL		
	Total	100 μL	_ µL		
Transfect cells	Replace growth me	edium on cells with 100 μ L o	f transfection mixture		

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24-well protocol Day 1 Cell plating Seed cells at a density that gives 70-90% confluency on the next day Day 2 Dilute sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl, pH 7.4 Prepare or Synthetic guide RNA working Dilute and mix crRNA and tracrRNA to a concentration working concentration of 2.5 µM in 10 mM solutions of Tris-HCl, pH7.4 materials for Dilute Edit-R CRISPR-Cas9 mRNA to a transfection Cas9 mRNA working concentration of 100 ng/µL in serum-free medium For mulitple wells For one well Combine working concentration Synthetic guide RNA 5 μL _ μL solutions for transfection Cas9 mRNA 10 µL _μL mixture Serum-free medium To 50 µL _μL Prepare working DharmaFECT Duo 0.5-0.8 μL _μL concentration transfection reagent DharmaFECT Serum-free medium To 50 μL $_{\mu L}$ Duo for transfection Combine Tube 1 and Tube 2 and carefully mix by pipeting Incubate at room temperature for 20 minutes before next step Prepare transfection Add full growth 400 µL _μL mixture medium 500 μL Total _μL

Replace growth medium on cells with 100 μL of transfection mixture

6-well protocol					
Day 1					
Cell plating	Seed cells at a density that gives 70-90% confluency on the next day				
Day 2					
Prepare working concentration solutions of materials for transfection	Synthetic guide RNA	Dilute sgRNA to a working concentration of 2 μ M in 10 mM Tris-HCl, pH 7.4 or Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 μ M in 10 mM Tris-HCl, pH7.4			
	Cas9 mRNA	Dilute Edit-R CRISPR-Cas9 mRNA to a working concentration of 100 ng/µL in serum-free medium			
		For one well	For mulitple wells		
Combine working	Tube 1				
concentration solutions for	Synthetic guide RNA	25 µL	_ μL		
transfection mixture	Cas9 mRNA	50 µL	_ μL		
	Serum-free medium	To 250 μL	_ μL		
Prepare working concentration DharmaFECT Duo for transfection	Tube 2				
	DharmaFECT Duo transfection reagent	2.5–20 μL	_ μL		
	Serum-free medium	Το 250 μL	_ μL		
	Incubate at room temperature for 5 minutes before next step				
Prepare transfection mixture	Combine Tube 1 and Tube 2 and carefully mix by pipeting				
	Incubate at room temperature for 20 minutes before next step				
	Add full growth medium	2,000 µL	_ µL		
	Total	2,500 μL	_ μL		
Transfect cells	Replace growth mediu	m on cells with 100 μL	of transfection mixture		

If you have any questions, contact

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Transfect cells

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