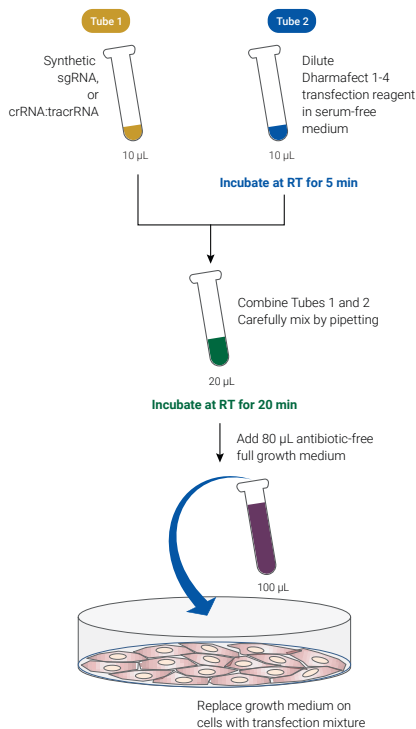


Edit-R synthetic guide RNA transfection protocol for Cas9 expressing cells

The following is an abbreviated protocol for transfecting synthetic guide RNA into cultured mammalian cells expressing Cas9 using DharmaFECT™ 1-4 transfection reagent (Cat. T-2001, T-2002, T-2003, T-2004). 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 [Technical Manual](#).

This protocol is written for transfection of cells into 96, 24, or 6-well tissue culture plates at 25 nM final concentration of synthetic guide RNAs.



96-well protocol

Day 1

Cell plating Seed cells at a density that is optimal for specific downstream phenotypic assay(s)

Day 2

Prepare working solutions of reagents for transfection Synthetic guide RNA Dilute sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl, pH 7.4 or Dilute and mix crRNA and tracrRNA to a working concentration of 2 µM in 10 mM Tris-HCl, pH 7.4

	For one well	For multiple wells	
Combine working solutions for transfection mixture	Tube 1		
	Synthetic guide RNA	1.25 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
Prepare working solution of Dharmafect 1-4 for transfection	Tube		
	DharmaFECT 1-4	0.05-0.8 µL	
	Serum-free medium	To 10 µL	_ µL
Incubate at room temperature for 5 minutes before next step			
Combine Tube 1 and Tube 2 and carefully mix by pipetting			
Incubate at room temperature for 20 minutes before next step			
Combine transfection mixture	Add full growth medium	80 µL	_ µL
	Total	100 µL	_ µL
	Transfect cells		
Replace growth medium on cells with 100 µL of transfection mixture			
Incubate cells for 72-96 hours before performing downstream phenotypic assay(s) or gene editing analysis			

24-well protocol

Day 1

Cell plating Seed cells at a density that is optimal for specific downstream phenotypic assay(s)

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, pH7.4

		For one well	For multiple wells
Combine working solutions for transfection mixture	Tube 1		
	Synthetic guide RNA	6.25 μ L	_ μ L
	Serum-free medium	To 50 μ L	_ μ L

		For one well	For multiple wells
Prepare working solution of Dharmafect 1-4 for transfection	Tube 2		
	DharmaFECT 1-4	0.24-4 μ L	_ μ L
	Serum-free medium	To 50 μ L	_ μ L

Incubate at room temperature for 5 minutes before next step

Combine Tube 1 and Tube 2 and carefully mix by pipeting

Incubate at room temperature for 20 minutes before next step

		For one well	For multiple wells
Combine transfection mixture	Add full growth medium	400 μ L	_ μ L
	Total	500 μ L	_ μ L

Transfect cells Replace growth medium on cells with 500 μ L of transfection mixture

Incubate cells for 72-96 hours before performing downstream phenotypic assay(s) or gene editing analysis

6-well protocol

Day 1

Cell plating Seed cells at a density that is optimal for specific downstream phenotypic assay(s)

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, pH7.4

		For one well	For multiple wells
Combine working solutions for transfection mixture	Tube 1		
	Synthetic guide RNA	25 μ L	_ μ L
	Serum-free medium	To 200 μ L	_ μ L

		For one well	For multiple wells
Prepare working solution of Dharmafect 1-4 for transfection	Tube 2		
	DharmaFECT 1-4	1-20 μ L	_ μ L
	Serum-free medium	To 250 μ L	_ μ L

Incubate at room temperature for 5 minutes before next step

Combine Tube 1 and Tube 2 and carefully mix by pipeting

Incubate at room temperature for 20 minutes before next step

		For one well	For multiple wells
Combine transfection mixture	Add full growth medium	1600 μ L	_ μ L
	Total	2000 μ L	_ μ L

Transfect cells Replace growth medium on cells with 2000 μ L of transfection mixture

Incubate cells for 72-96 hours before performing downstream phenotypic assay(s) or gene editing analysis

For more information

To find the contact information in your country for your technology of interest, please visit us at horizondiscovery.com/contact-us

Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom
©2022 Horizon Discovery Group Ltd and its affiliated companies. All rights reserved.