jetCRISPR[™] - RNP transfection reagent Short protocol - REVERSE

DAY 0: Cell preparation, RNP complex formation & transfection

1/ Prepare a cell suspension solution of V mL of serum containing medium according to the table below:

Quantities per well

Culture vessel	Number of cells per well	V volume of cell suspension solution
96-well	10 000 - 30 000	125 μL
24-well	80 000 - 120 000	500 μL
6-well	300 000 - 500 000	2 mL

Store cell suspension solution in the incubator while preparing RNP.

2/ Dilute gRNA in RNAse free water at 1 μ M and Cas9 protein in serum free medium at 1 μ M.

3/ In X μ L serum free medium, add Y μ L of gRNA and Y μ L of Cas9 protein (ratio 1:1) to prepare a RNP solution at 330 nM.

4/ Mix and incubate at room temperature for 10 min.

5/ Add W μL **jetCRISPR™** reagent into the RNP solution.

6/ Mix and incubate at RT for 15 min.

7/ Distribute Z μL of RNP transfection solution in each well and then add the cell suspension solution.

Quantities per well for a final RNP concentration of 30 nM

Cas9 pr	otein	gRNA	
4			
			Trypsinize, wash and
In serum free medium,	- T		resuspend cells in
add both Cas9 protein	- J.		their growth medium
and gRNA (ratio 1:1)			then growth medium
	4		1
	V		
Mix and incubate			
at RT for 10 min			
Add jetCRISPR™			
Mix and incubate	V		
at RT for 15 min			
Distribute the RNP transfection			
solution in each well and then add			
the cell suspension solution			
the cell suspension solution	*		
		100	
		1	
	1		
Incubate	e at 37°C	for 24 to 4	18 h



Culture vessel	X Volume of serum free medium (μL)	Y Volume of each gRNA and Cas9 protein (μL)	₩ Volume of jetCRISPR™ reagent (μL)	Z Volume of RNP transfection solution (μL)
96-well	4.3	4.1	0.3	12.5
24-well	17	16.5	1.2 (± 0.3)	50
6-well	68	66	4.8 (± 0.8)	200

DAY 1 or 2: Analyze the results

See back page for FORWARD protocol

Download complete protocol on http://www.polyplus-transfection.com/resources/product-literature/



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jetCRISPR[™] - RNP transfection reagent Short protocol - FORWARD

DAY 0: Cell seeding

1/ Seed cells in V mL of serum containing medium according to the table below:

Quantities per well

Culture vessel	Number of cells per well	V volume of growth medium during transfection
96-well	9 000 - 15 000	125 μL
24-well	40 000 - 60 000	500 μL
6-well / 35 mm	200 000 - 400 000	2 mL

DAY 1: RNP preparation & transfection

2/ Dilute gRNA in RNAse free water at 1 μM and Cas9 protein in serum free medium at 1 $\mu M.$

3/ In X μ L serum free medium, add Y μ L of gRNA and Y μ L of Cas9 protein (ratio 1:1) to prepare a RNP solution at 330 nM.

4/ Mix and incubate at room temperature for 10 min.

5/ Add W μL **jetCRISPR™** reagent into the RNP solution.

6/ Mix and incubate at RT for 15 min.

7/ Add Z μ L of RNP transfection solution onto the cells.

Quantities per well for a final RNP concentration of 30 nM

Culture vessel	X Volume of serum free medium (µL)	Volume of each gRNA and Cas9 protein (μL)	₩ Volume of jetCRISPR™ reagent (μL)	Z Volume of RNP transfection solution (µL)
96-well	4.3	4.1	0.3	12.5
24-well	17	16.5	1.2 (± 0.3)	50
6-well	68	66	4.8 (± 0.8)	200

DAY 2 or 3: Analyze the results

See back page for REVERSE protocol

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