

# 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 µM and Cas9 protein in serum free medium at 1 µM.

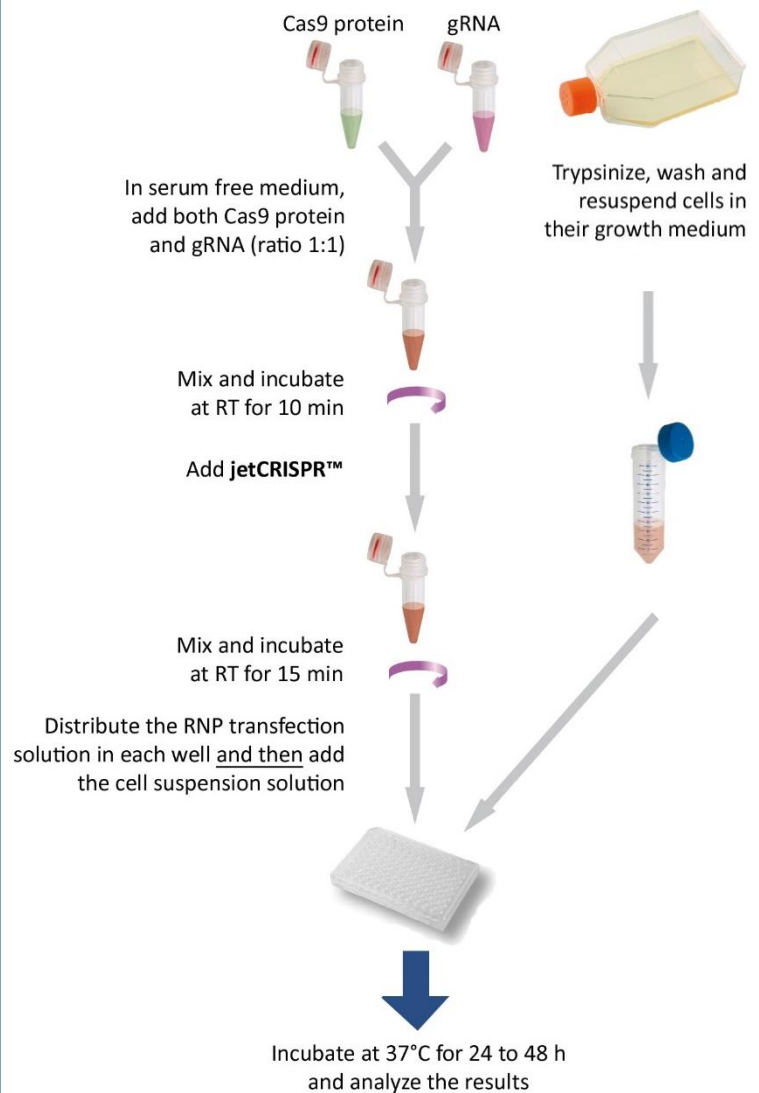
3/ In **X** µL serum free medium, add **Y** µL of gRNA and **Y** µL of Cas9 protein (ratio 1:1) to prepare a RNP solution at 330 nM.

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

Culture vessel	X Volume of serum free medium (µL)	Y Volume of each gRNA and Cas9 protein (µL)	W 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/>

# 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 µM.

3/ In **X** µL serum free medium, add **Y** µL of gRNA and **Y** µL 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** µL 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)	Y Volume of each gRNA and Cas9 protein (µL)	W 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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