jetCRISPR™
RNP transfection reagent
PROTOCOL

DESCRIPTION

jetCRISPR™ is a RiboNucleoProtein (RNP) transfection reagent designed to perform CRISPR-Cas9 genome editing in mammalian cells. This reagent has been specifically developed for efficient co-delivery of Cas9 Protein and guide RNA in a wide variety of targets. For optimal genome efficiency, we recommend using our SpCas9 Nuclease (Ref # 722-100).

Browse our cell transfection database to find optimized conditions according to your cell line:

http://www.polyplus-transfection.com/resources/cell-transfection-database/

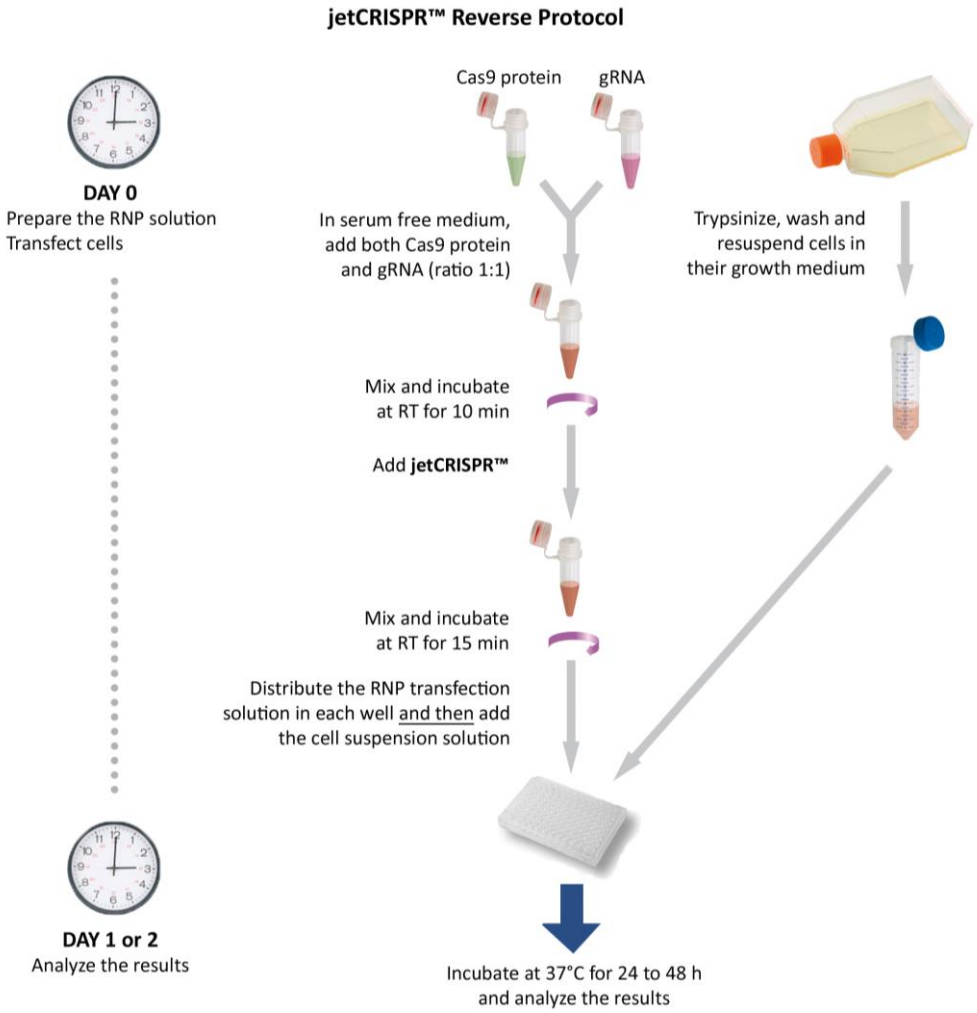
1 ABBREVIATIONS

- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
- Cas9: CRISPR Associated Protein 9 (endonuclease)
- crRNA: CRISPR RNA
- gRNA: guide RNA = sgRNA or tracrRNA+crRNA
- NLS sequence: Nuclear localization Signal sequence
- RNP: RiboNucleoProtein comprising Cas9 protein and gRNA (complex)
- RT: Room temperature
- sgRNA: Single guide RNA
- SpCas9: Streptoccocus pyogenes CRISPR Associated Protein 9 (endonuclease)
- tracrRNA: Trans-activating CRISPR RNA

2 REVERSE TRANSFECTION PROTOCOL

2.1 OVERVIEW

In the reverse protocol, RNP complexes are first deposited in each well, to which cells are subsequently added. The reverse transfection protocol is faster to perform in comparison to the forward transfection protocol, and is the recommended method of choice for efficient gene editing.



2.2 CELL PREPARATION

To prepare a cell suspension, trypsinize and wash cells with sterile 1X PBS solution. After centrifugation (5 min, 200 g, room temperature), resuspend cells in their growth medium (with serum and antibiotics if needed), according to the conditions recommended in Table 1. Store the cells in the incubator at 37°C while preparing the RNP transfection complexes.

jetCRISPR™ is stable in presence of serum and antibiotics. You may therefore use serum and antibiotic containing medium during the entire experiment.

Table 1. Recommended number of cells and volume of cell suspension to add per well format.

Culture vessel	Number of adherent cells per well	Surface area per well (cm^2)	Volume of cell suspension (mL)
96-well	10 000 - 30 000	0.3	0.125
24-well	80 000 - 120 000	1.9	0.5
6-well / 35 mm	300 000 - 500 000	9.4	2

Note: Pre-coated plate with extracellular matrix components (eg. fibronectin, gelatin, collagen) may be required for some cell lines.

2.3 RNP COMPLEX FORMATION

As starting conditions, we suggest testing CRISPR-Cas9/guide RNA ribonucleoprotein (RNP) at a final concentration of 30 nM. We recommend using the Polyplus-transfection SpCas9 Nuclease (Ref # 722-100) for optimal genome editing efficiency.

Before use, thoroughly mix the stock of Cas9 protein by gently flicking the tube. Dilute the protein in serum free medium to a final concentration of 1 µM according to the conditions recommended in Table 2.

Notes:

- *The molecular weight of SpCas9 Nuclease is 162 000 g/mol*
- *The concentration of SpCas9 Nuclease is 10 µg/µL or 61.7 µM*
- *100 µg of SpCas9 nuclease = 617 pmol*

Table 2. Recommended quantity of Cas9 protein to add per well format.

Culture vessel	Amount of Cas9 protein per well	Concentration to prepare RNP	Final concentration in the well
96-well plate	4.13 pmol (669 ng)	1 µM	30 nM
24-well plate	16.5 pmol (2.67 µg)		
6-well plate	66 pmol (10.7 µg)		

The following protocol is given for transfection of RNP at 30 nM, per well of a 96-well plate. Please refer to Table 3 to adapt gRNA and Cas9 protein quantities for other plate formats.

1. Dilute gRNA at 1 µM in RNAse free water and Cas9 protein at 1 µM in serum free medium.
2. Prepare a RNP solution at **330 nM**, by diluting Cas9 protein and gRNA (comprising crRNA and tracrRNA, or sgRNA) at a 1:1 molar ratio in serum free medium. For this, in a microtube containing 4.3 µL of serum free medium, add 4.1 µL of Cas9 protein and 4.1 µL of gRNA (1:1 molar ratio).
3. Mix by gently pipetting up and down.
4. Incubate for 10 min at room temperature.

Notes:

- *To prepare RNP solution at other concentrations, please refer to: 4. Optimization guidelines.*
- *For efficient genome editing, we recommend using a Cas9 protein with one or several Nuclear Localization Signals (NLS) sequence(s) such as Polyplus-transfection SpCas9 Nuclease.*

Table 3. Recommended volume of gRNA and Cas9 protein to prepare a 330 nM RNP solution.

Culture vessel	Volume of serum free medium per well (µL)	Volume of 1 µM gRNA per well (µL)	Volume of 1 µM Cas9 protein per well (µL)	Final Volume per well (µL)
96-well plate	4.3	4.1	4.1	12.5
24-well plate	17	16.5	16.5	50
6-well plate	68	66	66	200

2.4 RNP TRANSFECTION PROTOCOL (REVERSE)

The following protocol is given for transfection of RNP at 30 nM, per well of a 96-well plate. For other culture formats, please refer to Table 4.

1. For each well to transfect, transfer 12.5 µL of RNP solution in a microtube.
2. Vortex jetCRISPR™ reagent for 5 sec and spin down before use.
3. Add 0.3 µl of jetCRISPR™ reagent into the microtube. Mix by pipetting up and down and briefly spin down.
4. Incubate for 15 min at room temperature to allow RNP transfection complexes to form.
5. Distribute the RNP transfection solution in each well and then add the cell suspension solution. The RNP transfection solution represents 10% of the cell volume and the final RNP concentration is 30 nM. Homogenize by gently pipetting up and down.
6. Incubate the plate at 37°C.
7. Analyze 24 or 48 h post-transfection.

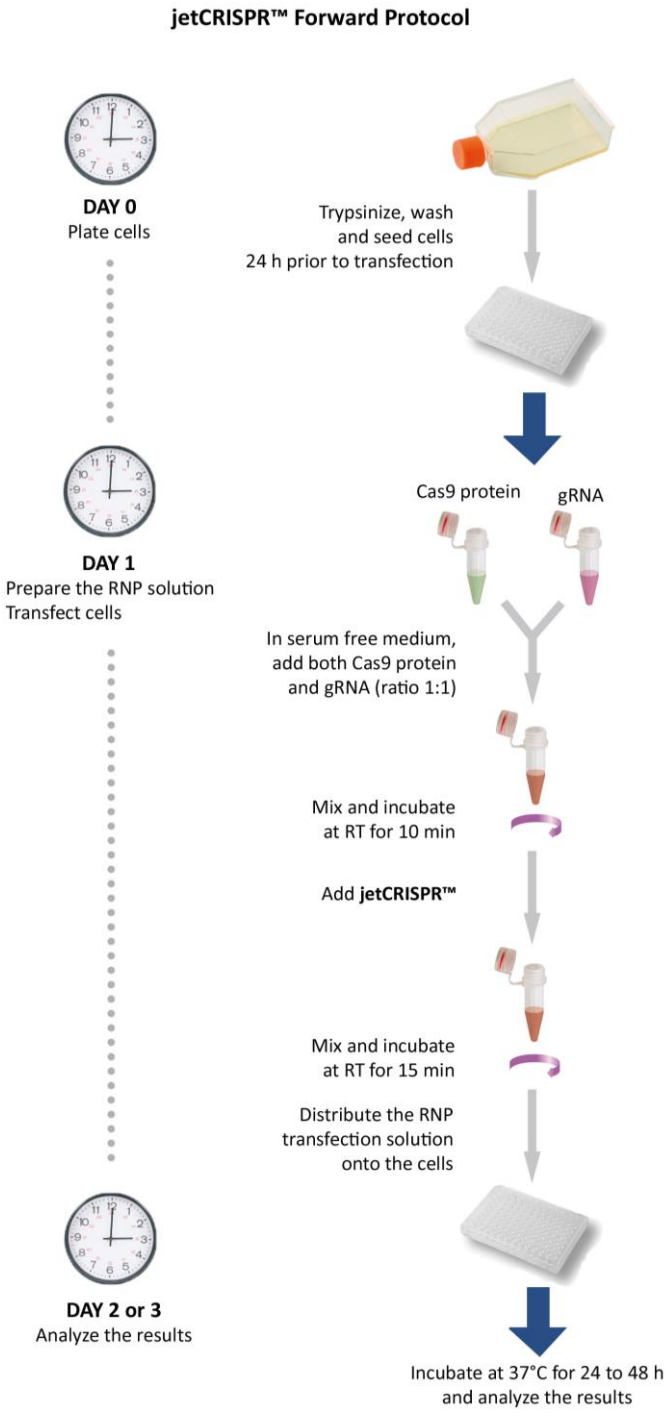
Table 4. RNP transfection guidelines according to the cell culture vessel format.

Culture vessel	Volume of RNP solution per well (µL)	Volume of jetCRISPR™ reagent per well (µL)	Volume of cell suspension per well (mL)
96-well	12.5	0.3	0.125
24-well	50	1.2	0.5
6-well	200	4.8	2

3 FORWARD TRANSFECTION PROTOCOL

3.1 OVERVIEW

This classical protocol allows transfection of sensitive adherent cells, such as primary cells or neurons.



3.2 CELL SEEDING

For optimal RNP transfection of adherent cells, we recommend seeding cells the day before transfection (Table 5) in their growth medium, with serum and antibiotics if needed. At the time of transfection, cells should reach 50 to 70% of confluency.

jetCRISPR™ is stable in the presence of serum and antibiotics. You may therefore use serum and antibiotic containing medium during the entire experiment.

Table 5. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well to seed the cells (mL)
96-well	9 000 - 15 000	0.3	0.125
24-well	40 000 - 60 000	1.9	0.5
6-well / 35 mm	200 000 - 400 000	9.4	2

3.3 RNP COMPLEX FORMATION

As starting conditions, we suggest testing CRISPR-Cas9/guide RNA ribonucleoprotein (RNP) at a final concentration of 30 nM. We recommend using the Polyplus-transfection SpCas9 Nuclease (Ref # 722-100) for optimal genome editing efficiency.

Before use, thoroughly mix the stock of Cas9 protein by gently flicking the tube. Dilute the protein in serum free medium to a final concentration of 1µM according to the conditions recommended in Table 6.

Notes:

- *The molecular weight of SpCas9 Nuclease is 162 000 g/mol*
- *The concentration of SpCas9 Nuclease is 10 µg/µL or 61.7 µM*
- *100 µg of SpCas9 nuclease = 617 pmol*

Table 6. Recommended quantity of Cas9 protein to add per well format.

Culture vessel	Amount of Cas9 protein per well	Concentration to prepare RNP	Final concentration in the well
96-well plate	4.13 pmol (669 ng)	1 µM	30 nM
24-well plate	16.5 pmol (2.67 µg)		
6-well plate	66 pmol (10.7 µg)		

The following protocol is given for transfection of RNP at 30 nM, per well of a 96-well plate. Please refer to Table 7 to adapt gRNA and Cas9 protein quantities for other plate formats.

1. Dilute gRNA at 1 µM in RNAse free water and Cas9 protein at 1 µM in serum free medium.
2. Prepare RNP complexes at a stock concentration of **330 nM**, by diluting Cas9 protein and gRNA (comprising crRNA and tracrRNA, or sgRNA) at a 1:1 molar ratio in serum free medium. For this, in a microtube containing 4.3 µL of serum free medium, add 4.1 µL of Cas9 protein and 4.1 µL of gRNA (1:1 molar ratio).
3. Mix by gently pipetting up and down.
4. Incubate for 10 min at room temperature.

Notes:

- *To prepare RNP solution at other concentrations, please refer to: 4. Optimization guidelines.*
- *For efficient genome editing, we recommend using a Cas9 protein with one or several Nuclear Localization Signals (NLS) sequence(s) such as the Polyplus-transfection SpCas9 Nuclease.*

Table 7. Recommended volume of gRNA and Cas9 protein to prepare a 330 nM RNP solution.

Culture vessel	Volume of serum free medium per well (µL)	Volume of 1 µM gRNA per well (µL)	Volume of 1 µM Cas9 protein per well (µL)	Volume final per well (µL)
96-well plate	4.3	4.1	4.1	12.5
24-well plate	17	16.5	16.5	50
6-well plate	68	66	66	200

3.4 RNP TRANSFECTION PROTOCOL (FORWARD)

The following protocol is given for transfection of RNP at 30 nM per well of a 96-well plate. For other culture formats, please refer to Table 8.

1. For each well to transfect, transfer 12.5 µL of RNP solution in a microtube.
2. Vortex jetCRISPR™ reagent for 5 sec and spin down before use.
3. Add 0.3 µl of jetCRISPR™ reagent into the microtube. Mix by pipetting up and down and briefly spin down.
4. Incubate for 15 min at room temperature to allow RNP transfection complexes to form.
5. Add the RNP transfection complexes solution onto the cells. Homogenize by gently swirling the plate. The RNP transfection solution represents 10% of the cell volume and the final RNP concentration is 30 nM.
6. Incubate the plate at 37°C.
7. Analyze 24 or 48 h post-transfection.

Table 8: RNP transfection guidelines according to the cell culture vessel.

Culture vessel	Volume of growth medium per well (mL)	Volume of RNP solution per well (µL)	Volume of jetCRISPR™ reagent per well (µL)
96-well	0.125	12.5	0.3
24-well	0.5	50	1.2
6-well	2	200	4.8

4 OPTIMIZATION GUIDELINES

Transfection conditions should be optimized for each cell type. You may adjust the number of cells, the final concentration of RNP between 10 and 50 nM and the volume of jetCRISPR™ transfection reagent. Please refer to the Tables 9 & 10 to adapt your protocol.

Table 9. Transfection optimization guidelines.

Culture vessel	Number of adherent cells to seed per well (Reverse)	Number of adherent cells to seed per well (Forward)	Volume of jetCRISPR™ reagent per well (µL)
96-well	10 000 - 30 000	9 000 - 15 000	0.3 - 0.4
24-well	80 000 - 120 000	40 000 - 60 000	0.9 - 1.5
6-well	300 000 - 500 000	200 000 - 400 000	4 - 5.6

Table 10. Recommended volume of gRNA and Cas9 protein to prepare the RNP solution.

Culture vessel	RNP final concentration (nM)	Volume of serum free medium per well (µL)	Volume of 1 µM gRNA per well (µL)	Volume of 1 µM Cas9 protein per well (µL)	Volume final per well (µL)
96-well plate	10	9.7	1.4	1.4	12.5
	30	4.3	4.1	4.1	
	50	7.9	2.3*	2.3*	
24-well plate	10	39	5.5	5.5	50
	30	17	16.5	16.5	
	50	31.7	9.2*	9.2*	
6-well plate	10	156	22	22	200
	30	68	66	66	
	50	126	37*	37*	

*For 50 nM RNP, prepare solutions of gRNA and Cas9 protein at 3 µM instead of 1 µM.

Specific conditions for different cell lines can also be found in our cell transfection database, following this link: http://www.polyplus-transfection.com/resources/cell-transfection-database/.

Notes:

- *The quality and the structure of the Cas9 protein used for transfection can drastically affect the genome editing efficiency. Please consider testing several Cas9 proteins such as the Polyplus-transfection SpCas9 Nuclease to select the optimal one in your hands.*
- *To ensure high genome editing efficiency and to limit off-target effects, we recommend testing several gRNA sequences.*
- *For DNA insertion, we recommend first delivering the donor plasmid with jetPRIME® reagent (Ref # 114-XX) then the RNP solution with jetCRISPR™ reagent. Please contact our technical support for more information at:* support@polyplus-transfection.com.

5 TROUBLESHOOTING

Observations	Actions
Low genome editing efficiency	Optimize the volume of jetCRISPR™ reagent and the amount of RNP added per well. Increase the volume of jetCRISPR™ reagent first; if insufficient, increase the amount of RNP according to Table 8.
	Ensure that all reagents are RNAse free.
	Ensure that the quality of your gRNA is optimal (concentration and design).
	Test another Cas9 protein, such as the Polyplus-transfection SpCas9 Nuclease, with a different structure including NLS sequences.
	Ensure that your read-out is optimal (gRNA extraction, amount and quality, PCR amplification conditions, optimal primers, mismatch DNA endonuclease assay conditions, gel electrophoresis conditions, …).
Cellular toxicity	Decrease the amount of RNP added per well.
	Ensure the modified or edited gene does not induce toxicity.
	Decrease the volume of jetCRISPR™ according to Table 9.
	Optimize cell density according to Table 9.
	Change medium 4 hours after transfection.
Off-target effect	Optimize gRNA design.
	Decrease the amount of RNP added per well.
	Optimize the gene editing detection assay conditions (PCR amplification and endonuclease assay conditions).

6 PRODUCT INFORMATION

6.1 ORDERING INFORMATION

Product	Ref #	Vial size
jetCRISPR™	502-01	0.1 mL
	502-07	0.75 mL
	502-15	1.5 mL
SpCas9 Nuclease	722-100	100 µg

6.2 RELATED PRODUCTS

We provide a full range of reagents for all your CRISPR experiments:

- jetCRISPR™ for RNP delivery – Protein and guide RNA co-delivery
- jetPRIME® for CRISPR DNA plasmid approaches
- jetMESSENGER™ for RNA transfection (guide RNA and mRNA cotransfection)
- *in vivo*-jetPEI® for *in vivo* gene editing through DNA delivery

in vitro / *in vivo*	Introduction of the Cas9 by	Introduction of the Guide RNA by	Our solutions
in vitro	Protein	RNA	**jetCRISPR™** RNP transfection reagent
in vitro	DNA	DNA	**jetPRIME®** DNA transfection reagent
in vitro	mRNA	RNA	**jetMESSENGER™** mRNA transfection reagent
in vivo	DNA/mRNA	DNA/RNA	***in vivo*-jetPEI®** *in vivo* delivery reagent

6.3 CONTENT

1.5 mL of jetCRISPR™ transfection reagent is sufficient to perform up to 1250 transfections in 24-well plates or 300 transfections in 6-well plates.

100 µg of SpCas9 nuclease is sufficient to perform up to 37 transfections in 24-well plates, or 10 transfections in 6-well plates.

6.4 REAGENT USE AND LIMITATIONS

All Polyplus-transfection® reagents are developed, designed and sold exclusively for *in vitro* research purposes and non-human *in vivo* laboratory applications. They have not been tested for drug development, nor are they suitable for administration in humans.

6.5 QUALITY CONTROL

Every batch of jetCRISPR™ reagent is tested in-house in HEK-293 cells following a reverse protocol in 96-well plates with 30 nM of RNP (SpCas9 Nuclease & gRNA targeting the HPRT1 gene) using 0.3 µL of jetCRISPR™. INDEL (%) is quantified 48 hours post-transfection using the T7 endonuclease assay. Each vial is provided with a Certificate of Analysis.

Every batch of SpCas9 nuclease is tested by an in vitro activity assay using gRNA and linearized DNA target. Each vial is provided with a Certificate of Analysis.

6.6 FORMULATION AND STORAGE

jetCRISPR™ is shipped with an ice pack and should be stored at +5 ± 3°C upon arrival to ensure long term stability. jetCRISPR™ is stable for at least 6 months (502-01) to at least one year (other packaging sizes) when stored appropriately, as guaranteed and indicated on the Certificate of Analysis

SpCas9 nuclease is shipped at -20°C, and should be stored at -20 ± 5°C upon arrival to ensure long term stability. Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution and customer support.

6.7 TRADEMARKS

Polyplus-transfection and jetCRISPR are registered trademarks of Polyplus-transfection.

How to cite us: “jetCRISPR™ (Polyplus-transfection S.A, Illkirch, France)”

6.8 TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

Contact the friendly Polyplus technical support *via*:

- The Polyplus website: www.polyplus-transfection.com
- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87