Purified Cas9 mRNA, capped and polyA-tailed Application Guide

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Description

CRISPR/Cas9 allows scientists to do genome editing at will. Plasmid vectors may not be effective for some challenging cells and immune cells (such as monocytes and macrophages), because of the delivery issue or plasmid DNA degradation. Cas9 mRNA together with gRNA (invitro transcribed or synthesized) has been proven to be more effective in genome editing. In addition, the use of RNA over plasmid constructs avoids complications with promoter-embryo compatibility as well as the possibility of random integration of Cas9 nuclease and gRNA-expressing plasmids into the host genome.

Cas9 mRNA allows multiplex genome editing, where multiple target gene sequences can be edited simultaneously in a single transfection reaction by adding multiple gRNAs. Ready-to-use Cas9 mRNA makes gene editing versatile and simple, and changing target specificity only requires a change in the gRNA design.

OriGene's ready-to-use Cas9 mRNA is generated through *in-vitro* transcription and it has been capped and polyA-tailed. It mimics a fully processed mature mRNA. Additionally, Cas9 mRNA has a C terminal fusion Myc/DDK tag (DDK is the same as Flag tag) which can be used for detection, and purification of the Cas9 protein. Following transfection, the Cas9 protein is directed by gRNA to target specific genomic locus.

Shipping/Storage condition/Shelf Life:

Shipping on dry ice, store at or below -80°C. If stored properly, Cas9 mRNA is stable for 6 months.

Handling:

Thaw mRNA on ice. Upon first use, please spin briefly before opening and aliquot into single use portions. Do not vortex. Use only certified RNase-free reagents and consumables with proper RNase-free technique. Avoid freeze/thaw cycles.

Related Products:

Description	Catalog No.
Cas9 Protein	TP790148
Viromer mRNA**, transfection reagent for mRNA**	TT100313
CRISPR Vectors	
Cas9 antibodies	

CRISPR Products and Services

OriGene provides reagents and kits to support a broad range of CRISPR/Cas9 genome editing applications. For more information, visit https://www.origene.com/products/gene-expression/crispr-cas9 and our online CRISPR manual at https://cdn.origene.com/assets/documents/crispr-cas9/crispr_manual.pdf

Synthetic Single Guide RNA (sgRNA)

Genome editing with CRISPR-Cas9 genomic editing technology requires a guide RNA (gRNA) in order to cleave genomic DNA at a target sequence of interest.

Recently, synthetic single guide has been recognized as the preferred way for highly efficient and accurate editing. OriGene's synthetic single guide gRNA is a pure 100-mer RNA oligo that contains the target gRNA sequence and the tracrRNA scaffold in a single entity.

Benefits of synthetic sgRNA (100mer):

- **Better in vivo stability, n**o worry for RNase carry-over from the source
- **Better efficiency**
  Up to 90% genome editing efficiency
- **Cost-effective**
  Highly scalable for large numbers of experiments

To achieve best result in your gene editing experience, the synthetic single Guide RNA is highly recommended.

For more information, visit https://www.origene.com/products/gene-expression/crispr-cas9/sgrna

Validation Data

We have tested the expression and functionality of the ready-to-use Cas9 mRNA with sgRNA for their ability to cleave specific genomic sequences (Figure 1).

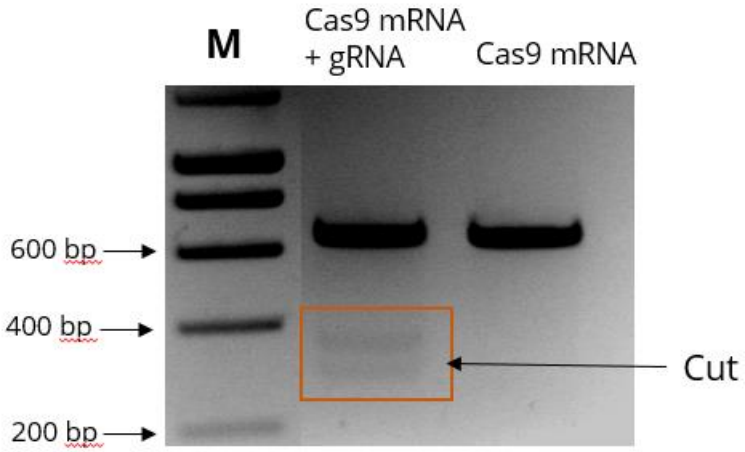

Figure 1. **Cas9 mRNA is functional in cells.** Cas9 mRNA was transfected into HEK 293T Cells with or without sgRNA. After 48 h, Genomic DNA was isolated from cells using Quick-DNA (Zymo Research) extraction solution. Gene editing (insertions/deletions) was measured by T7 Endonuclease I (T7E1, EnGen® Mutation Detection Kit, NEB) according to the recommended protocol.

Note: T7 Endonuclease I only recognize indel (Insertion and deletion) of ≥2 bases and its activity is sensitive to the DNA enzyme ratio, as well as incubation temperature and time. The results of this analysis may significantly underrepresent the total gene editing.

Experimental Protocol of transfecting Cas9 mRNA and sgRNA in HEK293T Cells

This standard transfection protocol is based on Viromer mRNA transfection reagent. Although the transfection protocols below have been proven to result in highly efficient transfection, it is encouraged to carefully optimize the reaction conditions for each individual cell type.

1. Cell Plating

Approximately 18-24 hours before transfection, plate ~3 X 10^5 adherent cells in 2 ml culture medium into each well of a 6-well plate or ~5x10^5 suspension cells per well to obtain 50-70% confluence on the following day. The amount of cells varies depending on the size of your cells.

2. Transfection complex formation (perform this step immediately before transfection)

a). In a small sterile tube, dilute mRNA in buffer mRNA to 11 ng/ul. For 6-well plate, 2 ug RNA is recommended. Then add 200ng of gRNA into the diluted mRNA.

b). In another sterile tube dilute Viromer mRNA according to figure 2. For 6-well plate, dilute 0.8 ul of Viromer mRNA into 19.2 ul of buffer mRNA.

c). Combine diluted mRNA from step a into diluted Viromer mRNA from step b, mix and incubate at room temperature for 15 min.

3. Add the transfection mixture onto cells drop wise. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate at 37°C for 24-48 hours.

Note: With Viromer mRNA, no medium change is necessary. If you wish to remove the complex, remove the medium 4-24 hours post-transfection and replace with fresh complete medium.

Figure 2. mRNA transfection summary

well format

		96-well	24-well	6-well	
Step 1: Dilute RNA	mRNA/ gRNA	0.1 ug/ 0.01 ug	0.5 ug/ 0.05 ug	2 ug/0.2 ug	
	Buffer mRNA in uL	9	45	180	
Step 2:	Viromer® mRNA in µl	0,04	0,2	0,8	**Always add buffer to Viromer®, not vice versa!** vortex 3-5s
	Buffer mRNA in µl	0,96	4,8	19,2	
Step 3:	complexation	Pipette the mRNA from step 1 into the diluted Viromer® of step 2. Mix swiftly and incubate 15 min at room temperature.			
	Transfer x µl of complexes into the wells.	10 µl 100 ng/well	50 µl 500 ng/well	200 µl 2 µg / well	
Step 4:	Forward transfection: Add transfection complexes onto the cells seeded a day before. Mix carefully.				
	Reverse Transfection: Add transfection complexes to empty wells and seed the cells (100µl) immediately afterwards. Mix carefully.				
Step 5:	Read-out Incubate cells as usual. There is no need to change medium unless high amounts of transfection complex cause toxicity. Monitor effects 12-24 hours post-transfection and determine the best conditions for your special cells. Note: mRNA expression can start as early as 6h after transfection.				

Microinjections and in vivo applications

While we haven't tested the use of Cas9 mRNA in microinjections or other in vivo–mediated delivery methods for transgenic animal model generation. It is well-established in the literature that transgenic animals can be generated via microinjection of Cas9 mRNA and guide RNA (sgRNA). The use of RNA over plasmid constructs avoids complications with promoter-embryo compatibility as well as the possibility of random integration of nuclease and gRNA-expressing plasmids into the host animal genome. Typical microinjection concentrations used in the literature are in the ranges of 20-200 ng/µl for Cas9 mRNA and 10-50 ng/µl for gRNA.

FAQ

Q: What's the advantage of using Cas9 mRNA over plasmid constructs?

The use of RNA over plasmid constructs avoids complications with promoter-cell compatibility as well as the possibility of random integration of Cas9 nuclease and gRNA-expressing plasmids into the host genome.

Q: What's the typical concentration of Cas9 mRNA for embryo microinjection?

Typical microinjection concentrations used in the literature are in the ranges of 20-200 ng/µl for Cas9 mRNA and 10-50 ng/µl for gRNA.

References:

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3. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013, 9; 153(4):910-918.
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