sgRNA Synthesis Kit, spCas9

Cat. No. G520

Store at -20°C.

Product Description

The **sgRNA Synthesis Kit, spCas9** is a complete kit **designed for high yield *in vitro* RNA transcription utilizing T7 RNA Polymerase**. This kit includes all reagents required to generate a DNA template and perform RNA transcription with subsequent DNA removal. Resulting products can be used directly for downstream CRISPR/Cas9 studies and/or RNA experiments. This kit contains reagents sufficient for 10 reactions generating up to 100 µg RNA per reaction.

Product Component	Quantity	Part No.
Scaffold Template and Primer Mix	100 µl	P990-1
2X sgRNA Synthesis Buffer	100 µl	P990-2
sgRNA Synthesis Enzyme Mix	50 µl	P990-3
MegaFi™ Pro Fidelity 2X PCR MasterMix	1.25 ml	P887-1
DNase I (RNase-Free)	20 µl	P091-1
10X DNase I Reaction Buffer	1.0 ml	P114
Nuclease-Free H_2O	1.0 ml	P100

Additional Materials Required (not included)

sgRNA Target-Specific Oligo

1M EDTA (Optional)

Target-Specific Oligo for sgRNA synthesis

The sgRNA *in vitro* transcription template is generated via oligo annealing and extension using the included **Scaffold Template and Primer Mix** and user-provided oligo with the following sequence:

T7 Promoter - sgRNA target sequence (20 nt) - Scaffold Overlap:
TTCTAATACGACTCACTATAGGGNNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAG

The Scaffold and Template Primer Mix, when combined with the Target-Specific Oligo, produces a DNA Template for the transcription of sgRNA specific to spCas9. **abm** offers sgRNA Target-Specific Oligo Design & Synthesis Service (Cat. No. **C337**). Resuspend the DNA oligo to 100 µM then dilute to 10 µM and store aliquots at -20°C.

Template Considerations

Synthetic DNA oligonucleotides, PCR products, or linearized plasmid DNA that contains a **double-stranded T7 promoter** region upstream of the template sequence can serve as a template for *in vitro* transcription. For efficient transcription, the initiating nucleotides must be one to three Gs. Termination can be achieved either using a terminator sequence or by designing templates for run-off transcription. *Note that the SP6 and T3 promoters are not compatible with this kit.*

Protocol

in vitro transcription reactions should be assembled in an RNase-free environment. The use of clean, automatic pipettes and filtered tips is recommended.

sgRNA Template Generation

1. Prepare the following reaction:

Product Component	Volume
MegaFi™ Pro Fidelity 2X PCR MasterMix	12.5 µl
Scaffold Template and Primer Mix	1 µl
sgRNA Target-Specific Oligo (10 µM)	1 µl
Nuclease-Free H_2O	10.5 µl
Total	25 µl

2. Perform PCR amplification as follows:

Step	Temperature	Time	Cycle
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	35
Annealing	55°C	15 sec	
Extension	72°C	15 sec	
Final Extension	72°C	5 min	1

3. Run 5 µl of PCR product on a 2% agarose gel and expect 130 bp amplicons. Store at -20°C or proceed to next step.

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in vitro Transcription

1. Thaw all reagents on ice. Mix each solution gently.
2. Prepare the following 20 µl reaction:

Product Component	Volume
2X sgRNA Synthesis Buffer	10 µl
sgRNA Synthesis Enzyme Mix	4 µl
DNA Template	1-5 µl*
Nuclease-Free H_2O	up to 20 µl

*Volume can be adjusted depending on PCR efficiency.

3. Mix gently and incubate the reaction at 37°C for 30 minutes (the duration of the reaction can be increased up to 4 hours or overnight for longer templates >500 bp).
4. After incubation, the transcribed RNA product can be used directly in downstream applications, stored at -20°C for up to six months, or stored at -80°C for longer-term. The transcribed RNA product can be treated with DNase I to remove DNA template as described below.

DNase I Treatment *(optional)*

1. To remove template DNA, the transcribed RNA product may be treated with DNase I by preparing the following reaction:

Product Component	Volume
Transcribed RNA product	20 µl
10X DNase I Reaction Buffer	10 µl
DNase I (RNase-Free)	2 µl (4 U)
Nuclease-Free H_2O	up to 100 µl

2. Incubate the reaction at 37°C for 15 minutes.
3. To inactivate DNase I activity, add 1 µl of 1M EDTA (not included) and incubate at room temperature for at least 10 minutes or purify with Column-Pure RNA Miniprep Kit (Cat. No. **D518**)
4. Products can be used directly in downstream applications, stored at -20°C for up to 6 months, or stored at -80°C for longer-term.

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