HyperScribe™ One-Step sgRNA Synthesis Kit

Product description

The CRISPR/Cas9 system is a widely used genome editing technology, which uses Cas9 nuclease to cut specific sites of targeted sequences of genomic DNA under the guidance of sgRNA, also known as gRNA (guide RNA). gRNA is a fusion product consisting of 18-20 bp CRISPR RNA (crRNA) sequences that complement the target gene sequences, and Trans-activating crRNA (tracrRNA) sequences that bind to Cas9 nucleases.In the cell, gRNA bound to Cas9 nuclease and guided Cas9 nuclease to cut the target gene at the upstream position of the proto-spacer adjacent motif (PAM) sequence, causing frameshift mutations in the target gene locus and eventually resulting in deletion mutations of the target gene.

This kit is a One-step method for the synthesis of sgRNA based on PCR amplification and in vitro transcription dependent on T7 RNA polymerase. The kit provides a sgRNA reaction mix, including a Scaffold Template sequence and a downstream primer for amplification of the sequence. Users only need to design and synthesize the Target-specific DNA oligo (i.e. the upstream primer) to obtain the corresponding sgRNA through this kit. After purification, the synthesized sgRNA can be co-transfected with Cas9 mRNA (No.R1015), or gene edited in cells expressing Cas9 protein, or mixed with Cas9 nuclease for enzyme digestion identification experiment. The one-step method for synthesizing sgRNA is simpler to operate compared to the Two-Step method, However, the yield is lower than that of the two-step method. If the two-step method for synthesizing sgRNA is required, it is recommended to use the HyperScribe™ two-step sgRNA Synthesis Kit (No. K1421).

Composition and storage conditions

Size / Components	20 rxns	Storage
2× sgRNA reaction mix	200 µL	-20°C
T7 RNA polymerase mix	40 µL	-20°C
DNase I (2 U/µL)	40 µL	-20°C

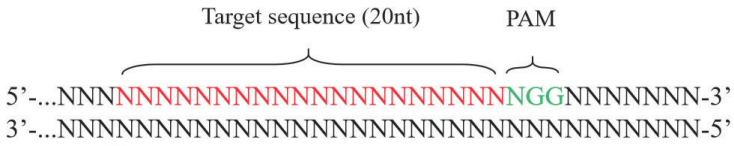
RNase-free H_2O	400 μL	-20°C

Shipping: Dry Ice **Shelf life:** 1 years

Experimental operation

1. Target DNA sequence selection and the design of PCR upstream primers (i.e. Target-specific DNA oligo)

(1) The 20 nt sequence adjacent to PAM (NGG) was selected as the target sequence of CRISPR/Cas9 system.



(2) PCR upstream primers (Target-specific DNA oligo) consist of four parts: a) T7 promoter sequence; b) The transcription initiation site needs to add 0-2 G; c) 20 nt sgRNA target sequence; d) 14 nt annealing sequence of Scaffold Template.

***Note:** The T7 promoter requires at least 2 G for effective transcription, and the number of G added depends on the 5' end of the target sequence. If the 5' end of the selected target sequence already contains 2 G, no additional G needs to be added to the transcription initiation site; If it contains 1 or 0 G, 1 or 2 additional G need to be added accordingly.

2. Synthesis of sgRNA

(1) Thaw the corresponding components, and then add the components in the order referenced to the table below to prepare the reaction system on the ice.

Reagent	Volume	Final Concentration
2× sgRNA reaction mix	10 μL	1×
Target-specific DNA Oligo (50 μM)	1 μL	2.5 μM final
T7 RNA polymerase mix	2 μL	
RNase-free H_2O	7 μL	
Total Reaction Volume	20 μL	

(2) Gently mix the reaction system (gentle suction or slight vortex), and then instantaneously centrifuge to collect residual liquid from the tube wall.

*Note: The operation process must be carried out strictly in accordance with the RNA operation specifications to avoid RNase contamination.

(3) Incubate at 37 ℃ for 2-4 h. Generally, the longer the reaction time, the more sgRNA is produced.

(4) After the reaction, add 2 μL of DNase I (2 U/μL) to the system and mix well. Incubate at 37 ° C for 30 min to fully digest the DNA template.

(5) Purify the transcripts. The sgRNA can be purified using RNA Clean Beads (No. K1812) or phenol chloroform extraction and ethanol precipitation.

(6) The product quality can be analyzed by RNA gel electrophoresis.

Notes

1. Organic solvents such as phenol and chloroform remaining in DNA will affect transcription efficiency.
2. It is recommended to design at least three different gRNAs for each target gene. Using multiple gRNAs at the same time increases the chances of editing success.
3. Strict attention should be paid to avoid RNase contamination during the experimental operation.
4. This product is for scientific use only.

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