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Express CRISPR sgRNA Synthesis Kit

(Cat# K1253-25; 25 Reactions; Store at -20°C)

I. Introduction:

BioVision's Express CRISPR sgRNA Synthesis Kit (*S. pyogenes*) is a neat handy tool for generating sgRNAs for spCas9 system. The 2X Express sgRNA Synthesis SuperMix contains all the components required for the *in vitro* transcription including an oligo template of the tracer sgRNA. To set up the reaction, simply combine the 2X Express sgRNA Synthesis SuperMix, OneScribe Enzyme Mix, DNA Polymerase, the target-specific oligo, and water in a 20 µl reaction. The target-specific oligo first anneals with the sgRNA scaffold complementary oligo included in the 2X Express sgRNA Synthesis SuperMix, and DNA extension will be performed by the DNA polymerase. The newly extended dsDNA will then serve as the template for sgRNA transcription. High quality sgRNA can be obtained in as little as 30 minutes. The synthesized sgRNA can be used directly in downstream applications such as sgRNA *in vitro* screening. An optional DNase I treatment followed by spin column purification can be performed to remove DNA template, and leftover NTPs and dNTPs. This optional step could result in a more suitable sgRNA for downstream *in vivo* studies and applications.

II. Application:

- sgRNA *in vitro* studies
- sgRNA *in vitro* screening

III. Key Features:

- Easy 1-step setup
- Short reaction time, only 30 mins
- RNaseOFF ribonuclease inhibitor included in the reagents for extra protection

IV. Kit Contents (25 rxns):

Components	Quantity	Part Number
2X Express sgRNA Synthesis SuperMix	250 µl	K1253-25-1
DNA Polymerase	10 µl	K1253-25-2
sgRNA Control Oligo (10 µM)	10 µl	K1253-25-3
OneScribe Enzyme Mix	100 µl	K1253-25-4
DNase I, RNase-Free (2 U/µl)	100 µl	K1253-25-5
10X DNase I Reaction Buffer	500 µl	K1253-25-6
Nuclease-free H_2O	1 ml	K1253-25-7

V. Shipment and Storage:

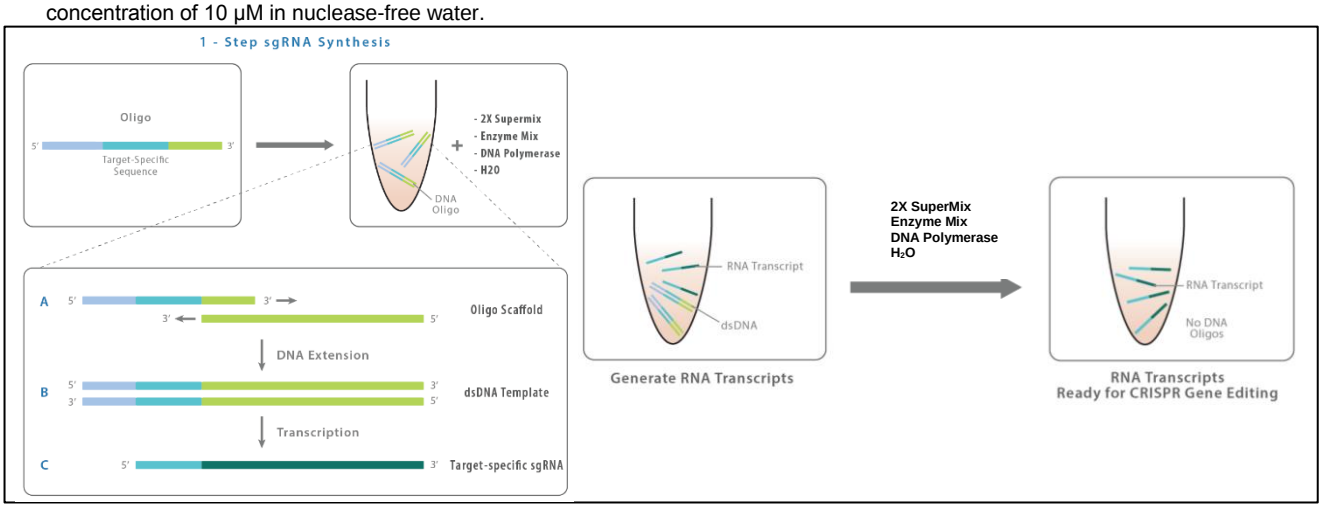
All the reagents are shipped at -20°C. Store all components at -20°C in a non-defrost cycle freezer. All the components are stable for 2 years from the date of shipment when stored and handled properly.

VI. Protocol:

Target-Specific Oligo Design:

sgRNA *in vitro* transcription template is generated via oligo annealing and extension using the complementary sgRNA Scaffold Template included in the reaction supermix. Follow the guidelines below to design your primer containing the T7 promoter sequence, the sgRNA sequence to the target of interest, followed by a Scaffold Template-specific sequence (refer to image below).

1. All DNA targeting sequence ends with the proto-spacer adjacent motifs (PAM) sequence, NGG, at its 3'end. However, only the 20 nucleotides upstream of the PAM sequence will be used as the sgRNA sequence, and the PAM sequence itself is NOT part of the sgRNA sequence.
2. The target-specific oligo contains the following 3 components of sequences as illustrated below: A T7 promoter sequence (23 nt at the 5' end of the primer which inserts "GGG" at the 5' end of the sgRNA and these three "G"s are crucial for efficient T7 transcription), a 20 nt actual sgRNA targeting sequence, and a Scaffold Template-specific sequence.
3. The target-specific oligo is 66 nt long and it should be subjected to salt-free purification following synthesis, and diluted to a concentration of 10 µM in nuclease-free water.



Reaction Setup:

All experiments should be performed in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross-contamination. The use of "clean" pipettes designated for RNA work and aerosol-resistant barrier tips is recommended.

1. Thaw DNA templates and all reagents on ice. Mix each solution by vortexing gently.
2. Prepare the following 20 µl reaction on ice in this order:

Product Components	**Quantity**
Nuclease-free H_2O	4.6 µl
2X Express sgRNA Synthesis SuperMix	10 µl
Target-Specific* or Control Oligo (10 µM)	1 µl
DNA Polymerase	0.4 µl
OneScribe Enzyme Mix	4 µl
Total Volume	**20 µl**

**** Target-Specific Oligo is not provided in the kit***

3. Mix contents of tube and centrifuge briefly.
4. Incubate reaction tube at 37°C for 30 mins to transcribe sgRNA; a longer incubation time of 45 mins may increase the yield of sgRNA up to 15%.
5. After incubation, products can be used directly in downstream applications, or stored. Store at -20°C for up to six months, and at -80°C for long-term. Products can also be treated with DNase I for DNA template removal as described in the protocol below.

DNase I Treatment (optional):

1. To remove template DNA, the transcription reaction from step 5 (above) may be treated with DNase I by preparing the following reaction:

Product Components	**Quantity**
sgRNA (step 5 above)	20 µl
Nuclease-free H_2O	66 µl
10X DNase I Reaction Buffer	10 µl
DNase I, RNase-Free (2 U/µl)	4 µl (8 U)
Total Volume	**100 µl**

2. Incubate the reaction at 37ºC for 15 mins followed by spin column purification.
3. After incubation, the products can be used directly in downstream applications, stored at -20ºC for up to six months, and stored at -80ºC for long-term.

VII. Related Products:

BV Product Name	**BV Cat. No.**
Gene Snipper™ Cas9 Proteins	M1094-M1103
Gene Snipper™ SaCas9 Proteins	M1280-M1283
Gene Snipper™ Cas9 GFP Proteins	M1284-M1286
CRISPR Genomic Cleavage Detection Kit	M1250-25
CRISPR sgRNA Screening Kit	M1251-25
Classic CRISPR sgRNA Synthesis Kit	M1252-25
Express CRISPR sgRNA Synthesis Kit	M1253-25

FOR RESEARCH USE ONLY! Not to be used on humans.