DATA SHEET

Jena Bioscience

HighYield T7 sgRNA Synthesis Kit (*Sp*Cas9)

Cloning-free preparation of *Sp*Cas9-specific sgRNA by *in vitro* transcription

Cat. No.	Amount
RNT-105	50 reactions

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months after date of delivery

Description:
HighYield T7 sgRNA Synthesis Kit (*Sp*Cas9) is designed for cloning-free synthesis of *Sp*Cas9-specific single-guided RNAs (sgRNAs) via *in vitro* transcription. SgRNAs direct sequence-specific DNA recognition once complexed with *Streptococcus pyogenes* Cas9 (*Sp*Cas9)[1,2]. The resulting sgRNA/*Sp*Cas9 ribonucleoprotein (RNP) complex can thus be used for site-specific cleavage, nicking or binding of dsDNA both *in vitro* and in living cells depending on the choice of *Sp*Cas9 variant (e.g. wildtype, nickase (D10A), nuclease deficient(D10A/H840A)). Cleavage, nicking or binding of *Sp*Cas9 variant occurs upstream of the *Sp*Cas9-specific DNA recognition sequence 5'-NGG-3' (protospacer adjacent motif (PAM) sequence, N = any nucleotide base).

Cloning-free synthesis of sgRNA-encoding DNA template for T7 RNA Polymerase-mediated *in vitro* transcription is easily performed via PCR assembly with provided *Sp*Cas9 scaffold and T7 promotor containing PCR primer[2]. Only a target-specific oligonucleotide (approx. 60 nt) needs to be provided. Amplification is performed with Ultra DNA Polymerase (also known as Phusion High-Fidelity Polymerase) to ensure the highest sequence accuracy as well as blunt-end formation. The crude PCR mix can directly be used as template for *in vitro* transcription.

HighYield T7 sgRNA Synthesis Kit (*Sp*Cas9) contains sufficient reagents for **50 PCR assembly and *in vitro* transcription reactions**. Other (s)gRNA-encoding T7 DNA templates (e.g with a different scaffold or for different Cas endonucleases) can efficiently be *in vitro* transcribed with the HighYield T7 RNA Synthesis Kit (#RNT-101).

Content:
Ultra DNA Polymerase[*]
1x 30 µl (2U/µl) in storage buffer with 50% glycerol (v/v)
[*]also known as Phusion High-Fidelity Polymerase

Ultra DNA sgRNA Reaction Buffer
1x 600 µl (5x)

dNTP mix
1x 100 µl (10 mM)

T7fwd_sgRNA
1x 60 µl (100 µM)
5'-GGATCCTAATACGACTCACTATAG-3'

T7rev_sgRNA
1x 60 µl (100 µM)
5'-AAAAAAGCACCGACTCGG-3'

***Sp*Cas9 scaffold**
1x 60 µl (1 µM)
5'-AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA
CGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC-3'

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HPRT control oligo
1x 15 µl (1 µM)

HighYield T7 RNA Polymerase Mix
3x 40 µl incl. RNase inhibitor and 50 % glycerol (v/v)

HighYield T7 Reaction Buffer
1x 200 µl (10x), HEPES-based

ATP - Solution
1x 100 µl (100 mM)

GTP - Solution
1x 100 µl (100 mM)

CTP - Solution
1x 100 µl (100 mM)

UTP - Solution
1x 100 µl (100 mM)

PCR-grade water
2x 1.2 ml

DTT
2x 100 µl (100 mM)

To be provided by user
Target-specific oligo
RNA purification tools
RNAse-free DNAse I

1. Prevention of RNAse contamination
Although a potent RNase Inhibitor is included, creating a RNAse-free work environment and maintaining RNAse-free solutions is critical for performing successful *in vitro* transcription reactions. We therefore recommend

- to perform all reactions in sterile, RNAse-free tubes using sterile pipette tips.
- to wear gloves when handling samples containing RNA.
- to keep all components tightly sealed both during storage and reaction procedure.

2. Design of target-specific oligonucleotide

- For more detailed information, please refer to our background information on target-specific oligonucleotide design: www.jenabioscience.com/images/741d0cd7d0/Target-specific_oligonucleotide_design_for_RNT-105.pdf

3. Synthesis of sgRNA-encoding T7 DNA template

- Prepare a 100 µM stock solution of target-specific oligonucleotide stock solution (see 2.) with PCR-grade water.
- Assemble PCR reaction on ice, voretex and spin down briefly.

Component	Volume	Final conc.
PCR-grade water	X µl	
Ultra DNA sgRNA Reaction Buffer (5x)	10 µl	1x
dNTP mix (10 mM)	1 µl	0.2 mM
T7fwd_sgRNA (100 µM)	1 µl	2 µM
T7rev_sgRNA (100 µM)	1 µl	2 µM
*Sp*Cas9 scaffold (1 µM)	1 µl	0.02 µM
Target-specific oligo (1 µM) (see 2.) alternatively HPRT control oligo	1 µl	0.02 µM
Ultra DNA Polymerase[*] (2 U/µl)	0.5 µl	1 U
Total volume	**50 µl**	

[*]also known as Phusion High-Fidelity DNA Polymerase

- Perform PCR reaction using the following cycling conditions.

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- Analyse 5 µl of PCR reaction mix on a 2% agarose gel. The expected product size is 127 nt.
- Purification is not required for subsequent *in vitro* transcription.

Cycle step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1x
Denaturation Annealing Elongation	95°C 57°C 72°C	20 sec 20 sec 20 sec	30x
Final Elongation	72 °C	2 min	1x

4. sgRNA Synthesis via *in vitro* transcription

The protocol is set up for 5 µl PCR mix as sgRNA-encoding T7 DNA template (see 3), but individual optimization might be required. Purified T7 DNA templates from different sources can be used as well (1-2 pmol per 20 µl reaction).

- Place HighYield T7 RNA Polymerase Mix on ice.
- Thaw all remaining components at room temperature (RT), mix by voretexing and spin down briefly.
- Assemble all components at RT to a nuclease-free microtube (sterile pipette tips) in the following order:
- Mix PCR-grade water, HighYield T7 Reaction Buffer and DTT by voretexing and spin down briefly.
- Add nucleotide solutions and sgRNA-encoding DNA template (e.g. 5 µl PCR mix from section 3), vortex and spin down briefly.
- Add HighYield T7 RNA Polymerase Mix, vortex and spin down briefly.
- Incubate for 2h at 37°C in the dark (e.g. PCR cycler). Depending on the RNA sequence individual optimization may increase product yield (0.5h–4h at 37°C).
- Analyse 1 µl of *in vitro* transcription reaction on a 2% agarose gel.

Component	Volume	Final conc.
PCR-grade water	3 µl	
HighYield T7 Reaction Buffer (10x)	2 µl	1x
DTT (100 mM)	2 µl	10 mM
ATP (100 mM)	1.5 µl	7.5 mM
UTP (100 mM)	1.5 µl	7.5 mM
CTP (100 mM)	1.5 µl	7.5 mM
GTP (100 mM)	1.5 µl	7.5 mM
PCR reaction mix (see 3.)	5 µl	
HighYield T7 RNA Polymerase Mix	2 µl	
Total volume	**20 µl**	

Please note: Reagents for the following steps are not provided within this kit.

DNA template removal

Depending on the down-stream application, removal of template DNA might be required. We recommend a salt-resistant, high efficiency DNAase such as Turbo™DNAse (ThermoFisher). Follow the manufacturer instructions.

Removal of 5'-triphosphate groups

5'-ends of *in vitro* phosphorylated RNAs carry a triphosphate group that is known to trigger RIG-1 mediated innate immune response in mammalian cells[3,4]. Removal with phosphatases (e.g. CIP) before final purification is therefore recommended for RNA probes intended for transfection experiments. Please refer to the following references for more detailed information: [3],[4].

RNA purification

Purification of RNA is required for certain applications such as RNA concentration mesurement. Spin column purification will remove proteins, salts and unincorporated nucleotides. Please follow the manufacturer instructions and ensure that the columns match with product size and possess a sufficient binding capacity (e.g. ≥ 50 µg RNA Clean & Concentrator™ columns (Zymo Research) or Monarch® RNA Cleanup kit (NEB)). Other RNA purification methods such as LiCl precipitation may work but have not been tested.

RNA quantitation

RNA concentration can be determined by absorbance measurement at 260 nm (A_{260}) according to the Law-of-Lambert-Beer (A_{260} = 1 corresponds to 40 µg/ml ssRNA).

Related Products:

HighYield T7 RNA Synthesis Kit, #RNT-101

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Selected References:

[1] Jinek *et al.* (2012) A programmable dual-RNA guided DNA Endonuclease in adaptive bacterial immunity. *Science* **337**:816.

[2] Modzelewski *et al.* (2018) Efficient mouse genome engineering by CRISPR-EZ technology. *Nature Protocols* **13 (6)** :1253.

[3] Wienert *et al.* (2018) In vitro transcribed guide RNAs trigger an innate immune response via RIG-I pathway. *PLoS Biol.* **16 (7)** :e2005840.

[4] Kim *et al.* (2018) CRISPR RNAs trigger innate immune responses in human cells. *Genome Res.* **28 (3)**:367.