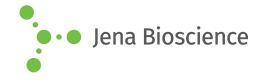
DATA SHEET





HighYield T7 RNAi Kit

dsRNA synthesis for RNAi applications via T7 RNA Polymerase-mediated in vitro transcription

Cat. No.	Amount
RNT-134	50 reactions x 20 μl

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 RNAi Kit is designed to produce large amounts of double-stranded RNA (dsRNA) > 200 bp via T7 RNA Polymerase-mediated *in vitro* transcription. The resulting dsRNA can subsequently be used for RNA interference (RNAi) applications. Preparation of small interfering dsRNA, known as siRNA (approximately 20 bp long) is principally feasible as well however, we recommend a chemical siRNA synthesis approach for short dsRNAs.

The kit contains sufficient reagents for 50 reactions of 20 μ l each (7.5 mM each NTP). A 20 μ l reaction yields > 40 μ g dsRNA (> 2 mg/ml) after 30 min incubation (1 μ g each T7 control template I & II, 0.5 kbp RNA transcripts). Yields may however vary depending on the template (promotor design, sequence length, secondary structure formation).

Content:

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

1x 1.2 ml

DTT

1x 150 µl (100 mM)

DNase I Solution

1x 60 μl (1 u/μl)

RNase A Solution

1x 200 μ l (4 mg/ml)

Sodium Acetate Solution

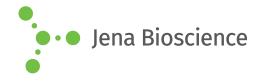
1x 1 ml (3 M)

T7 G-initiating control template I (0.5 kbp)

10 μl (0.5 μg/μl)



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■ HighYield T7 RNAi Kit

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T7 G-initiating control template II (0.5 kbp)

10 μl (0.5 μg/μl)

To be provided by user

T7 Promotor-containing DNA template Isopropanol 70 % Ethanol

Important Notes (Read before starting)

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.

Template requirements

 Template type: Linearized plasmid DNA or PCR products containing a double-stranded T7 class II phi2.5 or class III phi6.5 promotor region upstream of the target sequence.

Minimum T7 promotor sequences:

T7 class III phi6.5 promotor 5'-TAATACGACTCACTATA**G**NN...-3' Bold: First base incorporated into RNA, NN: ideally CG

or

T7 class II phi2.5 promotor 5'-TAATACGACTCACTATT**A**NN...-3' Bold: First base incorporated into RNA, NN: ideally GG

· Template strategies:

Option 1:

Prepare two identical DNA templates with a single T7 promoter at opposite ends of the region to be transcribed. *In vitro* transcription reactions of each template can be performed to obtain complementary ssRNA molecules. An additional annealing step is required. Alternatively, transcribe both templates simultaneously in one reaction. dsRNA and unspecific aggregates are most often directly generated in this case.

Aggregate formation can be solved by performing an additional denaturation and annealing step.

Option 2:

Prepare one DNA template with opposite T7 promoter at the 5' ends of each strand. dsRNA and unspecific aggregates are most often directly generated during *in vitro* transcription. Aggregate formation can be solved by performing an additional denaturation and annealing step.

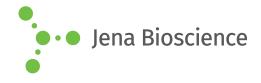
Template quality: DNA template quality directly influences yield
and quality of transcription reaction. Linearized plasmid DNA
needs to be fully digested and to be free of contaminating
RNase, protein and salts. We recommend selecting restriction
enzymes that generate blunt ends or 5'-overhangs and purification by phenol/chloroform extraction. A PCR mixture can be
used directly however, better yields will usually be obtained
with purified PCR products (e.g. via silica-membrane based purification columns).

In vitro Transcription protocol

The protocol is optimized for 1 μg DNA template (refer to "Important Notes" regarding template requirements).

- 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 template DNA, vortex and spin down briefly.
- Add HighYield T7 RNA Polymerase Mix vortex and spin down briefly.
- Incubate for 0.5h 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).

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Component	Volume	Final conc.
PCR-grade water	Xμl	
HighYield T7 Reac- tion 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
Template DNA	Xμl	1 μg
HighYield T7 RNA Polymerase Mix	2 μl	
Total volume	20 μl	

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 dsRNA).

Annealing/dsRNA preparation

An annealing step is recommended independent of the template strategy. Successfull annealing depends on nearly equimolar amounts of ssRNA. If ssRNA from separate *in vitro* transcription reactions are annealed (see "Template strategies, Option 1"), first check on transcription efficiency of each ssRNA by agarose gelelectrophoresis.

- Mix equal volumes of complementary ssRNA in case of similar transcription efficiency or adjust volume ratios accordingly.
- Incubate at 70 °C for 10 min and cool down slowly to room temperature (approximately 20 min)
- Do not place the reaction on ice. Annealing of the dsRNA occurs during cool down.

DNA template and ssRNA removal

- Dilute RNase A Solution 1:200 with PCR-grade water (prepare dilution freshly each time).
- Pipette 1 μl diluted RNase A Solution and 1 μl DNase I to 20 μl in vitro annealing reaction.
- Incubate for 30 min at 37 °C.
- Discard the diluted RNase Solution

dsRNA Purification

- Add 0.1 volume of 3 M Sodium Acetate (pH 5.2) and 1 volume of isopropanol to dsRNA reaction.
- Mix and place on ice for 5 min.
- Spin 10 min at top speed in a microcentrifuge. A white pellet should be visible.
- Carefully pipett off the supernatant and wash the pellet with 500 µl of 70 % ethanol by swiveling the reaction tube.
- Completely pipett off the ethanol and dry the pellet for 15 min with the tube open.
- Resuspend dsRNA in 2-5 fold of inital volume with PCR-grade water.