T7 *In Vitro* Transcription Kit esiSCRIBE
100 Reactions (10 µl each)
Catalog-#: T7-esiSCRIBE-100

Storage: -20°C

Content

100 µl	Reaction buffer, 10x
1 ml	RNase-free water
20 µl	Reference template (100 ng/µl)
300 µl	NTP mix, 25 mM each U, G, C, ATP
100 µl	T7 enzyme mix

Composition of reaction buffer, 10x (pH 8.0 at 20°C):
400 mM Tris-HCl
100 mM Dithiothreitol
200 mM $MgCl_2$

Application

The kit is intended for the production of RNA by *in vitro* transcription utilizing the enzyme T7 RNA polymerase. T7 RNA polymerase transcripts from DNA containing a T7 promoter sequence (5'-GC TAA TAC GAC TCA CTA TA**G GGA GA**-3'; bold: transcriptional start site; underlined: optional sequence tag known to increase RNA yield).

To obtain transcripts of defined length, linear template DNA should be used, which may be generated by linearization of plasmid DNA, by PCR or by hybridization of synthetic oligonucleotides.
The kit is suitable for the production of single-stranded RNA molecules, such as guide (g)RNA for CRISPR/Cas9 experiments and hybridization probes for FISH (fluorescence in-situ hybridization), RNA SELEX (systematic evolution of ligands by exponential enrichment), Northern blots etc., or the generation of double-stranded (ds)RNA for RNA interference (RNAi).
Ready-to-transfect esiRNAs (endoribonuclease-prepared siRNAs) and gRNAs are also available from Eupheria Biotech (www.eupheria.com).

Reaction Conditions

This kit contains all necessary components to run 100 reactions of 10 µl each. Typically, ~20 µg of RNA can be produced from a 10-µl reaction. The reference (ref.) template is dsDNA with two T7 promoters at both termini that yields dsRNAs of 480 bp in length. It is recommended to perform a control reaction with the provided reference template to verify proper reaction conditions and handling.
In general, various factors may influence RNA yield. An important factor is the purity of the DNA template. PCR products should be purified by anion exchange chromatography (e.g. QIAquick PCR Purification Kit (Qiagen)) or by phenol/chloroform extraction before transcription.
Plasmid DNA is suitable without further purification if extracted by e.g. the QIAprep Spin Miniprep Kit (Qiagen). Otherwise, a phenol/chloroform extraction followed by precipitation and resuspension in water should be performed.
Also consider that transcription products are sensitive to degradation by RNase. Therefore, ensure that all components (including template) are free of RNase contamination. Always wear gloves when handling RNA or kit components and use filter tips only.

Transcription reaction setup (total volume of 10 µl):

NTP mix (25 mM each)	3 µl
Reaction buffer, 10x	1 µl
Template (~ 500 ng)	up to 5.0 µl
T7 enzyme mix	1 µl
RNase-free water	add to 10 µl

Mix the reaction well, spin down and incubate for 2 – 4 hours at 37°C. For difficult templates, yields can be increased by overnight incubation.
If dsRNA is needed (e.g. for RNAi experiments), we recommend the following temperature profile on a PCR cycler to anneal the complementary single-stranded RNAs from the transcription reaction:
3 min at 90°C; ramp rate 0.1°C/sec to 70°C; 3 min at 70°C; ramp rate 0.1°C/sec to 50°C; 3 min at 50°C; ramp rate 0.1°C/sec to 20°C.

Optional

To decrease the risk of RNase activity in the reaction, RNase inhibitors may be used. In this case, include 1 µl (40 U/µl) of RNase OUT (Invitrogen) or similar reagents in the reaction mixture.
If desired, the DNA template can be removed by digestion with RNase-free DNase I. In this case, add DNase I to the reaction mixture right after transcription and incubate for an additional 30 min at 37°C. DNase I can be deactivated by heating for 5 min at 90°C, or be removed by phenol/chloroform extraction.

Troubleshooting

Low or no yield:
Carry out a control reaction using 4.5 µl of the reference template. The yield should be ~20 µg from a 10-µl reaction. Check the product size by agarose gel electrophoresis. One 480-bp dsRNA band is expected (see figure). If the control reaction worked, but the intended RNA cannot be generated, check if:

1. the template sequence is difficult (e.g. GC rich, repetitive elements).
Solution: Increase incubation time to 12 hours and decrease incubation temperature to 30°C.

2. the template DNA contains high concentrations of salt, EDTA or detergents.
Solution: Purify DNA by phenol/chloroform extraction, precipitation or perform exchange chromatography (e.g. QIAquick PCR Purification Kit (Qiagen)).

3. RNase contaminates the reaction mix.
Solution: Add RNase inhibitors to the reaction mixture. Make sure that all components used are RNase free (e.g. pipets, tips, tubes, all buffers, including gel running buffer and sample loading buffer). Also, sometimes the contamination originates from the DNA template. Therefore, purify the template before transcription as outlined under 2.

Appearance of abortive products:
Transcription of long RNAs (> 300 nt) may lead to abortive products in addition to the full-length product. This does not necessarily indicate RNase contamination but may be due to a cryptic termination signal in the template sequence and/or a rigid secondary structure of the nascent RNA.
Solution: Reduce reaction temperature to 30°C, or even down to 20°C and increase the incubation time to 12 hours.

Agarose gel (1.5%) analysis of 1 µl transcription product in 5 µl loading buffer (M: Marker; Lane 1: 480-bp dsRNA) produced by esiSCRIBE with the reference template.

Technical support:
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