



Highest quality & performance

All Fermentas products are manufactured in class D clean room facilities, qualified and certified as per EU directives and ISPE guidelines. Fermentas quality assurance is carried out according to ISO9001 quality and ISO14001 environmental management systems, guaranteeing batch-to-batch reproducibility. Integration of our clean room and ISO systems ensures stability and the absence of contaminants in all of our products.

In vitro TRANSCRIPTION

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Selection Guide

Applications	Procedures	Products to use	Cat. #	Page
Generation of template DNA for <i>in vitro</i> transcription	Cloning into vectors containing an RNA polymerase promoter	CloneJET™ PCR Cloning Kit (T7 promoter)	K1231/2	326
		InsTAclone™ PCR Cloning Kit* (T7 promoter)	K1213/4	328
		pTZ19R (T7 promoter)	SD0141	502
		FastDigest® Restriction Enzymes	FDXXXX	5
		Conventional Restriction Enzymes	ERXXXX	75
		FastAP™ Thermosensitive Alkaline Phosphatase	EF0651/2/4	242
		Shrimp Alkaline Phosphatase (SAP)	EF0511	243
		Rapid DNA Ligation Kit	K1422/3	329
		T4 DNA Ligase	EL0014	330
		Generation of PCR products	See chapter PCR, qPCR, RT-PCR & dNTPs	
	Plasmid purification	GeneJET™ Plasmid Miniprep Kit	K0502/3	345
	Linearization of plasmid DNA	FastDigest® Restriction Enzymes	FDXXXX	5
		Conventional Restriction Enzymes	ERXXXX	75
	Blunting of 3'-overhangs	T4 DNA Polymerase	EP0061/2	252
		dNTP Mix, 2 mM each	R0241/2	466
High yield <i>in vitro</i> transcription up to 200 µg RNA per 20 µl reaction	Unlabeled RNA and high specificity non-radiolabeled RNA probes	TranscriptAid™ T7 High Yield Transcription Kit	K0441	362
		Aminoallyl-UTP, 50 mM	R1091	471
		Pyrophosphatase, Inorganic	EF0221	272
Conventional <i>in vitro</i> transcription >10 µg RNA per 20 µl reaction	Unlabeled RNA and high specificity radiolabeled RNA probes	TranscriptAid™ T7 High Yield Transcription Kit	K0441	362
		T7 RNA Polymerase	EP0111/2/3	363
		T3 RNA Polymerase	EP0101/2/3	364
		SP6 RNA Polymerase	EP0131/3	365
		Pyrophosphatase, Inorganic	EF0221	272
		RiboLock™ RNase Inhibitor	E00381/2	271
		NTP Set, 100 mM	R0481	468
		ATP, 100 mM	R0441	469
		GTP, 100 mM	R0461	
		CTP, 100 mM	R0451	
	UTP, 100 mM	R0471	470	
	DEPC-treated Water	R0601/3	476	
RNA amplification	First strand cDNA synthesis	RevertAid™ Premium First Strand cDNA Synthesis Kit	K1651/2	302
		RevertAid™ H Minus First Strand cDNA Synthesis Kit	K1631/2	304
		RevertAid™ First Strand cDNA Synthesis Kit	K1621/2	306
		First Strand cDNA Synthesis Kit	K1611/2	308
		Maxima™ Reverse Transcriptase	EP0741/2/3	299
		RevertAid™ Premium Reverse Transcriptase	EP0731/2/3	301
		RevertAid™ H Minus Reverse Transcriptase	EP0451/2	303
		RevertAid™ Reverse Transcriptase	EP0441/2	305
		M-MuLV Reverse Transcriptase	EP0351/2	307
		AMV Reverse Transcriptase	EP0461	309
		RiboLock™ RNase Inhibitor	E00381/2	271
		dNTP Mix, 10 mM each	R0191/2/3	466
		DEPC-treated Water	R0601/3	476
	Second strand cDNA synthesis	DNA Polymerase I	EP0041/2	249
		RNase H	EN0201/2	267
High yield <i>in vitro</i> transcription	TranscriptAid™ T7 High Yield Transcription Kit	K0441	362	
Labeling of RNA	See chapter Molecular Labeling & Detection			
Removal of template DNA	DNase I, RNase-free	EN0521/3/5	256	
	0.5 M EDTA, pH 8.0	R1021	477	

* not available in certain countries.

(continued on next page)



Applications		Products to use	Cat. #	Page
Precipitation of RNA		3 M Sodium Acetate Solution, pH 5.2	R1181	478
		Glycogen, RNA grade	R0551	479
Analysis of RNA	RNA ladders	RiboRuler™ Low Range RNA Ladder	SM1831	442
		RiboRuler™ Low Range RNA Ladder, ready-to-use	SM1833	
		RiboRuler™ High Range RNA Ladder	SM1821	
		RiboRuler™ High Range RNA Ladder, ready-to-use	SM1823	
	Agarose	TopVision™ Agarose	R0491/2	484
		TopVision™ Low Melting Point Agarose	R0801	
	Electrophoresis buffers	10X TBE Buffer	B52	430
		50X TAE Buffer	B49	
Loading dye solution	2X RNA Loading Dye	R0641	443	
Blotting and detection	Hybridization	SensiBlot™ Plus Nylon Membrane	M1001/2	377
	Biotin detection	Biotin Chromogenic Detection Kit	K0661	377
		BCIP-T	R0821/2	483
		NBT	R0841/2	

6

Products

TranscriptAid™ T7 High Yield Transcription Kit

#K0441 for 50 reactions (of 20 µl)

Related Products

• CloneJET™ PCR Cloning Kit	p.326
• InstAclone™ PCR Cloning Kit	p.328
• pTZ19R DNA	p.502
• Rapid DNA Ligation Kit	p.329
• Aminoallyl-UTP	p.471
• NTP Set	p.468
• RiboLock™ RNase Inhibitor	p.271
• Pyrophosphatase, Inorganic (from yeast)	p.272
• DNase I, RNase-free	p.256
• RiboRuler™ RNA Ladders	p.442
• 2X RNA Loading Dye	p.443
• TopVision™ Agarose	p.484
• T4 RNA Ligase	p.240
• T4 Polynucleotide Kinase	p.244
• Alkaline Phosphatases	pp.242-243
• Water, nuclease-free	p.476

Description

The TranscriptAid™ T7 High Yield Transcription Kit is designed for high yield *in vitro* transcription from DNA templates containing a T7 RNA Polymerase promoter, including linearized plasmids or PCR products. The kit contains reagents for 50 reactions of 20 µl. Each reaction yields up to 200 µg RNA from 1 µg of template in 2 hours (see Fig. 6.1). The reaction can be scaled up to produce milligram amounts of full-length RNA. The kit can be used to produce both long and short transcripts for applications that require large yields of RNA. All necessary reagents for transcription are included, as well as the RiboRuler™ High Range RNA Ladder for sizing and quantification.

Features

- Exceptionally high yields – up to 200 µg in 2 hours.
- Versatile – suitable for both short and long RNA transcripts.
- Milligram amounts of RNA in a single, scaled-up reaction.
- Flexible – generates unlabeled, labeled or capped RNA.
- RiboRuler™ RNA Ladder supplied with kit for sizing and quantification.

Applications

- *In vitro* transcription.
- *In vitro* translation.
- Generation of hybridization probes for:
 - microarrays,
 - *in situ* hybridization,
 - blotting.
- RNase protection assays.
- RNA binding protein assays.
- Antisense RNA and RNAi.
- RNA amplification.
- Microinjection studies.

Quality Control

A 20 µl reaction containing 1 µg of the control template DNA generates 140-170 µg of intact 2.2 kb RNA transcript after a 2 h incubation.

Components of the Kit

- TranscriptAid™ Enzyme Mix
- 5X TranscriptAid™ Reaction Buffer
- DNase I, RNase-free
- ATP Solution
- CTP Solution
- GTP Solution
- UTP Solution
- Control template
- 3 M Sodium Acetate Solution, pH 5.2
- DEPC-treated Water
- 2X RNA Loading Dye Solution
- RiboRuler™ High Range RNA Ladder, ready-to-use
- 0.5 M EDTA, pH 8.0
- Detailed protocol

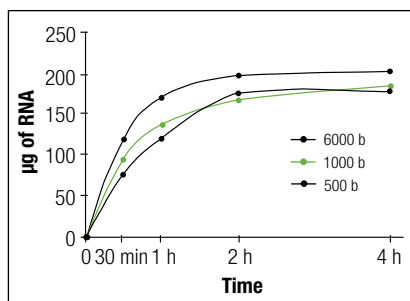


Figure 6.1. Time course of RNA synthesis. 500 b, 1000 b and 6000 b RNA transcripts were generated with TranscriptAid™ T7 High Yield Transcription Kit. 1 µg of DNA template was used in 20 µl reactions. Yields of RNA were determined at different transcription reaction time points using an Agilent 2100 Bioanalyzer.

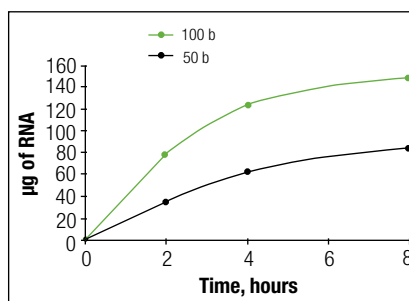


Figure 6.2. Time course of short RNA synthesis. 50 and 100 base RNA transcripts generated with TranscriptAid™ T7 High Yield Transcription Kit in 0-8 hour reaction time. RNA yield was evaluated with the Agilent 2100 Bioanalyzer.



Figure 6.3. High yield of full length RNA transcripts. 500, 1000 and 6000 base RNA transcripts were generated using high yield *in vitro* transcription reactions containing 1 µg of DNA template. Each 20 µl reaction was incubated for 2 hours at 37°C. The transcription products were diluted 80-fold and 6 µl was run on a 1% agarose/TAE gel supplemented with 0.5 µg/ml ethidium bromide.

M – RiboRuler™ High Range RNA Ladder, ready-to-use (#SM1823).

- 1, 4, 7** – 500 b RNA transcripts.
2, 5, 8 – 1000 b RNA transcripts.
3, 6, 9 – 6000 b RNA transcripts.



Protocols and Recommendations

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- » 6.2. Avoiding RNase contamination p.368
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T7 RNA Polymerase

#EP0111 5000 u (20 u/μl)

Supplied with:
5X Transcription Buffer 1.25 ml

#EP0112 5x5000 u (20 u/μl)

#EP0113 HC, 25000 u (≥100 u/μl)

Both Supplied with:
5X Transcription Buffer 5x1.25 ml

Related Products

- TranscriptAid™ T7 High Yield Transcription Kit p.362
- RiboLock™ RNase Inhibitor p.271
- DNase I, RNase-free p.256
- RiboRuler™ RNA Ladders p.442
- NTP Set p.468
- Aminoallyl-UTP p.471
- Pyrophosphatase, Inorganic (from yeast) p.272
- Glycogen, RNA grade p.479
- Agarase p.354
- 0.5 M EDTA, pH 8.0 p.477
- Water, nuclease-free p.476

Description

Bacteriophage T7 RNA Polymerase is a DNA-dependent RNA polymerase with strict specificity for its respective double-stranded promoter. The enzyme catalyzes the 5'→3' synthesis of RNA on either single-stranded DNA or double-stranded DNA downstream from the promoter.

Feature

Incorporates modified nucleotides (e.g., aminoallyl-, biotin-, fluorescein-, digoxigenin-labeled nucleotides).

Applications

Synthesis of unlabeled and labeled RNA that can be used:

- for hybridization (1), *in vitro* RNA translation (2);
- as arRNA (3), siRNA (4), substrate in RNase protection assays (5), template for genomic DNA sequencing (6);
- in studies of RNA secondary structure and RNA-protein interactions (7), RNA splicing (8).

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases is confirmed by appropriate tests. Functionally tested in *in vitro* transcription reaction.

Source

E. coli cells with a cloned gene encoding the T7 RNA Polymerase.

Molecular Weight

99 kDa monomer.

Concentration

20 u/μl
≥100 u/μl, HC

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction (adsorbed on DE-81) in 60 minutes at 37°C.

Enzyme activity is assayed in the following mixture: 40 mM Tris-HCl (pH 8.0), 6 mM MgCl₂, 10 mM DTT, 2 mM spermidine, 0.5 mM of each NTP, 0.6 MBq/ml [³H]-ATP, 20 μg/ml plasmid DNA containing the T7 promoter sequence.

Storage Buffer

The enzyme is supplied in:
50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM DTT, 0.1 mg/ml BSA, 0.03% (v/v) ELUGENT Detergent, 50% (v/v) glycerol.

5X Transcription Buffer

20 mM Tris-HCl (pH 7.9 at 25°C), 30 mM MgCl₂, 50 mM DTT, 50 mM NaCl, 10 mM spermidine.

Inhibition and Inactivation

- Inhibitors: metal chelators, enzyme activity is reduced by 50% at NaCl or KCl concentration above 150 mM.
- Inactivated by heating at 70°C for 10 min or by addition of EDTA.

Note

Consensus promoter sequence:

-15
-10
-5
+1
+5
 T7 TAATAC**CGACT**CACTATA**G**GGAGA

Position +1 indicates the first nucleotide incorporated into RNA during transcription. Only bases at positions +1 through +3 are critical for transcription, and they must be a G and 2 purine bases, respectively (9).

References

1. Melton, D.A., et al., Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter, *Nucleic Acids Res.*, 12, 7035-7056, 1984.
2. Krieg, P.A., Melton, D.A., Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs, *Nucleic Acids Res.*, 12, 7057-7070, 1984.
3. Melton, D.A., Injected antisense RNAs specifically block messenger RNA translation *in vivo*, *Proc. Natl. Acad. Sci. USA*, 82, 144-148, 1985.
4. Bernstein, E., et al., Role for bidentate ribonuclease in the initiation step of RNA interference, *Nature*, 409, 363-366, 2001.
5. Peebles, C.L., et al., A self-splicing RNA excises an intron lariat, *Cell*, 44, 213-223, 1986.
6. Church, G.M., Gilbert, W., Genomic sequencing, *Proc. Natl. Acad. Sci. USA*, 81, 1991-1995, 1984.
7. Witherell, G.W., et al., Cooperative binding of R17 coat protein to RNA, *Biochemistry*, 29, 11051-11057, 1990.
8. Krainer, A.R., et al., Normal and mutant human β-globin pre-mRNAs are faithfully and efficiently spliced *in vitro*, *Cell*, 36, 993-1005, 1984.
9. Jorgensen, E.D., et al., Specific contacts between the bacteriophage T3, T7, and SP6 RNA polymerases and their promoters, *J. Biol. Chem.*, 266, 645-651, 1991.



Protocols and Recommendations

- » 6.1. DNA template preparation for *in vitro* transcription p.368
- » 6.3. *In vitro* transcription p.368
- » 6.4. Synthesis of radiolabeled RNA probes of high specific activity p.368

T3 RNA Polymerase

#EP0101 2000 u (20 u/μl)

Supplied with:
5X Transcription Buffer 0.5 ml

#EP0102 10000 u (20 u/μl)

#EP0103 HC, 10000 u (≥100 u/μl)

Both supplied with:
5X Transcription Buffer 2x1.25 ml

Related Products

• RiboLock™ RNase Inhibitor	p.271
• DNase I, RNase-free	p.256
• RiboRuler™ RNA Ladders	p.442
• NTP Set	p.468
• Aminoallyl-UTP	p.471
• Pyrophosphatase, Inorganic (from yeast)	p.272
• Glycogen, RNA grade	p.479
• Agarase	p.354
• 0.5 M EDTA, pH 8.0	p.477
• DEPC-treated Water	p.476

Description

Bacteriophage T3 RNA Polymerase is a DNA-dependent RNA polymerase with strict specificity for its respective double-stranded promoter. The enzyme catalyzes the 5'→3' synthesis of RNA on either single-stranded DNA or double-stranded DNA downstream from the promoter. It incorporates modified nucleotides.

Feature

Incorporates modified nucleotides (e.g., aminoallyl-, biotin-, fluorescein-, digoxigenin-labeled nucleotides).

Applications

- Synthesis of unlabeled and labeled RNA that can be used:
- for hybridization (1), *in vitro* RNA translation (2);
 - as aRNA (3), siRNA (4), substrate in RNase protection assays (5), template for genomic DNA sequencing (6);
 - in studies of RNA secondary structure and RNA-protein interactions (7), RNA splicing (8).

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases is confirmed by appropriate tests. Functionally tested in *in vitro* transcription reaction.

Source

E. coli cells with a cloned gene encoding the T3 RNA Polymerase.

Molecular Weight

99 kDa monomer.

Concentration

20 u/μl,
≥100 u/μl, HC

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction (adsorbed on DE-81) in 60 minutes at 37°C.

Enzyme activity is assayed in the following mixture: 40 mM Tris-HCl (pH 8.0), 6 mM MgCl₂, 10 mM DTT, 2 mM spermidine, 0.5 mM of each NTP, 0.6 MBq/ml [³H]-ATP, 20 μg/ml plasmid DNA containing the T3 promoter sequence.

Storage Buffer

The enzyme is supplied in:
50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM DTT, 0.1 mg/ml BSA, 0.03% (v/v) ELUGENT Detergent, 50% (v/v) glycerol.

5X Transcription Buffer

200 mM Tris-HCl (pH 7.9 at 25°C), 30 mM MgCl₂, 50 mM DTT, 50 mM NaCl, 10 mM spermidine.

Inhibition and Inactivation

- Inhibitors: metal chelators, enzyme activity is reduced above 250 mM by 50% at NaCl or KCl concentration.
- Inactivated by heating at 70°C for 10 min or by addition of EDTA.

Note

Consensus promoter sequences:

```

      -15  -10  -5   +1  +5
T3  AATTAACCCCTCACTAAA GGAGA
  
```

Position +1 indicates the first nucleotide incorporated into RNA during transcription. Only bases at positions +1 through +3 are critical for transcription, and they must be a G and 2 purine bases, respectively (9).

References

1. Melton, D.A., et al., Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter, *Nucleic Acids Res.*, 12, 7035-7056, 1984.
2. Krieg, P.A., Melton, D.A., Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs, *Nucleic Acids Res.*, 12, 7057-7070, 1984.
3. Melton, D.A., Injected antisense RNAs specifically block messenger RNA translation *in vivo*, *Proc. Natl. Acad. Sci. USA*, 82, 144-148, 1985.
4. Bernstein, E., et al., Role for bidentate ribonuclease in the initiation step of RNA interference, *Nature*, 409, 363-366, 2001.
5. Peebles, C.L., et al., A self-splicing RNA excises an intron lariat, *Cell*, 44, 213-223, 1986.
6. Church, G.M., Gilbert, W., Genomic sequencing, *Proc. Natl. Acad. Sci. USA*, 81, 1991-1995, 1984.
7. Witherell, G.W., et al., Cooperative binding of R17 coat protein to RNA, *Biochemistry*, 29, 11051-11057, 1990.
8. Krainer, A.R., et al., Normal and mutant human β-globin pre-mRNAs are faithfully and efficiently spliced *in vitro*, *Cell*, 36, 993-1005, 1984.
9. Jorgensen, E.D., et al., Specific contacts between the bacteriophage T3, T7, and SP6 RNA polymerases and their promoters, *J. Biol. Chem.*, 266, 645-651, 1991.

For patent and license information see p.557



Protocols and Recommendations

- » 6.1. DNA template preparation for *in vitro* transcription p.368
- » 6.3. *In vitro* transcription p.368
- » 6.4. Synthesis of radiolabeled RNA probes of high specific activity p.368



SP6 RNA Polymerase

#EP0131 2000 u (20 u/μl)

Supplied with:
5X Transcription Buffer 0.5 ml

#EP0133 HC, 5000 u (≥100 u/μl)

Supplied with:
5X Transcription Buffer 1.25 ml

Related Products

• RiboLock™ RNase Inhibitor	p.271
• DNase I, RNase-free	p.256
• RiboRuler™ RNA Ladders	p.442
• NTP Set	p.468
• Aminoallyl-UTP	p.471
• Pyrophosphatase, Inorganic (from yeast)	p.272
• Glycogen, RNA grade	p.479
• Agarase	p.354
• 0.5 M EDTA, pH 8.0	p.477
• DEPC-treated Water	p.476

Description

Bacteriophage SP6 RNA Polymerase is a DNA-dependent RNA polymerase with strict specificity for its respective double-stranded promoter. The enzyme catalyzes the 5'→3' synthesis of RNA on either single-stranded DNA or double-stranded DNA downstream from the promoter. It incorporates modified nucleotides.

Feature

Incorporates modified nucleotides (e.g., aminoallyl-, biotin-, fluorescein-, digoxigenin-labeled nucleotides).

Application

Synthesis of unlabeled and labeled RNA that can be used:

- for hybridization (1), *in vitro* RNA translation (2);
- as aRNA (3), siRNA (4), substrate in RNase protection assays (5), template for genomic DNA sequencing (6);
- in studies of RNA secondary structure and RNA-protein interactions (7), RNA splicing (8).

Quality Control

The absence of endo-, exodeoxyribonucleases and ribonucleases confirmed by appropriate tests. Functionally tested in *in vitro* transcription reaction.

Source

E. coli cells with a cloned gene encoding the SP6 RNA polymerase.

Molecular Weight

99 kDa monomer.

Concentration

20 u/μl,
≥100 u/μl, HC

Definition of Activity Unit

One unit of the enzyme incorporates 1 nmol of AMP into a polynucleotide fraction (adsorbed on DE-81) in 60 minutes at 37°C.

Enzyme activity is assayed in the following mixture: 40 mM Tris-HCl (pH 8.0), 6 mM MgCl₂, 10 mM DTT, 2 mM spermidine, 0.5 mM of each NTP, 0.6 MBq/ml [³H]-ATP, 20 μg/ml plasmid DNA containing the SP6 promoter sequences.

Storage Buffer

The enzyme is supplied in:
50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM DTT, 0.1 mg/ml BSA, 0.03% (v/v) ELUGENT Detergent, 50% (v/v) glycerol.

5X Transcription Buffer

200 mM Tris-HCl (pH 7.9 at 25°C), 30 mM MgCl₂, 50 mM DTT, 50 mM NaCl, 10 mM spermidine.

Inhibition and Inactivation

- Inhibitors: metal chelators, enzyme activity is reduced by 50% at NaCl or KCl concentration above 150 mM.
- Inactivated by heating at 70°C for 10 min or by addition of EDTA.

Note

Consensus promoter sequences:

```

      -15  -10   -5   +1  +5
      |    |    |    |    |
SP6  ATTTAGGTGACACTATAGAAGNG
  
```

Position +1 indicates the first nucleotide incorporated into RNA during transcription. Only bases at positions +1 through +3 are critical for transcription, and they must be a G and 2 purine bases, respectively (9).

References

- Melton, D.A., et al., Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter, *Nucleic Acids Res.*, 12, 7035-7056, 1984.
- Krieg, P.A., Melton, D.A., Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs, *Nucleic Acids Res.*, 12, 7057-7070, 1984.
- Melton, D.A., Injected antisense RNAs specifically block messenger RNA translation *in vivo*, *Proc. Natl. Acad. Sci. USA*, 82, 144-148, 1985.
- Bernstein, E., et al., Role for bidentate ribonuclease in the initiation step of RNA interference, *Nature*, 409, 363-366, 2001.
- Peebles, C.L., et al., A self-splicing RNA excises an intron lariat, *Cell*, 44, 213-223, 1986.
- Church, G.M., Gilbert, W., Genomic sequencing, *Proc. Natl. Acad. Sci. USA*, 81, 1991-1995, 1984.
- Witherell, G.W., et al., Cooperative binding of R17 coat protein to RNA, *Biochemistry*, 29, 11051-11057, 1990.
- Krainer, A.R., et al., Normal and mutant human β-globin pre-mRNAs are faithfully and efficiently spliced *in vitro*, *Cell*, 36, 993-1005, 1984.
- Jorgensen, E.D., et al., Specific contacts between the bacteriophage T3, T7, and SP6 RNA polymerases and their promoters, *J. Biol. Chem.*, 266, 645-651, 1991.



Protocols and Recommendations

- » 6.1. DNA template preparation for *in vitro* transcription p.368
- » 6.3. *In vitro* transcription p.368
- » 6.4. Synthesis of radiolabeled RNA probes of high specific activity p.368

Related Products by Application

RIBONUCLEASE INHIBITION	Cat. #	Size	Applications	Page
RiboLock™ RNase Inhibitor, 40 u/μl	E00381	2500 u	• Protection of RNA from RNase A, B and C degradation at temperatures up to 55°C under a wide range of reaction conditions.	271
	E00382	4x2500 u		
	E00384	24x2500 u		



Protocols and Recommendations

- » 6.3. *In vitro* transcription p.368
- » 6.4. Synthesis of radiolabeled RNA probes of high specific activity p.368

TRANSCRIPTION ENHANCEMENT	Cat. #	Size	Supplied with	Applications	Page
Pyrophosphatase, Inorganic*, (from yeast), 0.1 u/μl	EF0221	10 u	Storage (Dilution) buffer 1 ml	• Increases the yield of <i>in vitro</i> transcription reactions by reducing the inhibitory effect of pyrophosphates.	272

* Use of this product in certain applications may be covered by patents and may require a license.

RNA TRANSCRIPT CLEAN-UP	Cat. #	Size	Supplied with	Applications	Page
DNase I, RNase-free, 1 u/μl	EN0521	1000 u	10X Reaction buffer with MgCl ₂ 1 ml 25 mM EDTA 1 ml	• Removal of template DNA after <i>in vitro</i> transcription reactions.	256
DNase I, RNase-free, 1 u/μl	EN0525	1000 u	10X Reaction buffer with MgCl ₂ 1 ml 10X Reaction buffer w/o MnCl ₂ 1 ml 100 mM MnCl ₂ 1 ml 25 mM EDTA 1 ml		
DNase I, RNase-free, 50 u/μl	EN0523	HC, 1000 u	10X Reaction buffer with MgCl ₂ 1 ml 25 mM EDTA 1 ml		
Agarase, 0.5 u/μl	E00461	100 u	–	• Recovery of RNA transcripts from low melting point (LM) agarose gels.	354



Protocols and Recommendations

- » 5.6. Removal of template DNA after *in vitro* transcription p.358

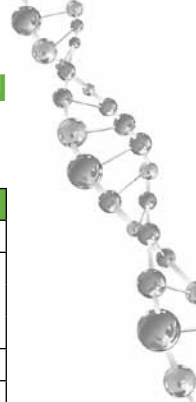
RNA LIGATION	Cat. #	Size	Supplied with	Applications	Page
T4 RNA Ligase, 10 u/μl	EL0021	1000 u	10X Reaction buffer 0.2 ml 10 mM ATP solution 0.2 ml 1 mg/ml BSA solution 0.2 ml	• Joining RNA to RNA. • RNA 3'-end labeling with cytidine 3', 5'-bis [α- ³² P] phosphate.	240

RNA TRANSCRIPT SIZING and QUANTIFICATION	Cat. #	Size	Supplied with	Applications	Page
RiboRuler™ Low Range RNA Ladder	SM1831	5x20 μl	2X RNA Loading Dye 1 ml	• RNA sizing and quantification on native or denaturing gels. • Northern blotting.	442
RiboRuler™ Low Range RNA Ladder, ready-to-use	SM1833	5x40 μl			
RiboRuler™ High Range RNA Ladder	SM1821	5x20 μl			
RiboRuler™ High Range RNA Ladder, ready-to-use	SM1823	5x40 μl			



Protocols and Recommendations

- » 10.1. General recommendations for RNA electrophoresis p.445
- » 10.2. Preparation of RNA ladders for electrophoresis p.445



NUCLEOTIDES	Cat. #	Size	Applications	Page
NTP Set, 100 mM	R0481	4x25 µmol	<ul style="list-style-type: none"> • <i>In vitro</i> transcription. • RNA amplification. • siRNA synthesis. • aRNA synthesis. 	468
ATP, 100 mM	R0441	25 µmol		469
CTP, 100 mM	R0451	25 µmol		470
GTP, 100 mM	R0461	25 µmol		471
UTP, 100 mM	R0471	25 µmol		
Aminoallyl-UTP, 50 mM	R1091	2.5 µmol	<ul style="list-style-type: none"> • Indirect non-radioactive enzymatic labeling of RNA during <i>in vitro</i> transcription. 	471

REACTION SETUP AND TERMINATION	Cat. #	Size	Applications	Page	
Water, nuclease-free	R0581	5x1 ml	<ul style="list-style-type: none"> • Resuspension and dilution of RNA samples or reaction mixtures. 	476	
	R0582	30 ml		476	
DEPC-treated Water	R0603	5x1 ml			
	R0601	30 ml			
0.5 M EDTA, pH 8.0	R1021	5x1 ml	<ul style="list-style-type: none"> • Termination of enzymatic reactions. 	477	

RNA PRECIPITATION	Cat. #	Size	Applications	Page
Glycogen, RNA grade	R0551	2x0.1 ml	<ul style="list-style-type: none"> • Precipitation of RNA from diluted solutions. 	479
3 M Sodium Acetate Solution, pH 5.2	R1181	1 ml	<ul style="list-style-type: none"> • Precipitation of RNA. 	478

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Protocols and Recommendations
 » 6.5. Purification of RNA transcripts p.369

RNA ELECTROPHORESIS	Cat. #	Size	Applications	Page
2X RNA Loading Dye	R0641	1 ml	<ul style="list-style-type: none"> • Loading of RNA on agarose or polyacrylamide gels. 	443
50X TAE Buffer	B49	1 litre	<ul style="list-style-type: none"> • Analytical electrophoresis of DNA templates and RNA transcripts. • Preparative electrophoresis of RNA transcripts. • Blotting assays. 	430
10X TBE Buffer	B52	1 litre		
TopVision™ Agarose	R0491	100 g	<ul style="list-style-type: none"> • Analytical electrophoresis of nucleic acids. • Preparative electrophoresis. • Blotting assays. 	484
	R0492	500 g		
TopVision™ Low Melting Point Agarose	R0801	25 g	<ul style="list-style-type: none"> • In-gel enzymatic processing experiments. • Analytical electrophoresis of nucleic acids. • Preparative electrophoresis using Agarose. 	

Protocols and Recommendations
 » 10.1. General recommendations for RNA electrophoresis p.445
 » 6.6. Evaluation of transcription reaction products p.369

Protocols and Recommendations

6.1. DNA template preparation for *in vitro* transcription

Double stranded linear DNA with blunt or 5'-protruding ends can be used as template for *in vitro* transcription. Linearized plasmid DNA, PCR products or cDNA can be used as templates for transcription if they contain a double-stranded RNA polymerase promoter region in the correct orientation.

Consensus promoter sequences of different RNA Polymerases:

	-15	-10	-5	+1
T7	TAATACGACTCACTATA	GGG		
T3	AATTAACCTCACTAAA	GGG		
SP6	ATTTAGGTGACACTATA	GAA		

G will be the first base (+1) of the RNA transcript

The synthesis of sense or antisense RNA transcripts depends on the orientation of the promoter with respect to the target sequence. The target sequence must be placed downstream of the promoter for sense RNA and must be inverted for antisense RNA transcription.

Plasmid Templates

Quality

Plasmid DNA quality affects transcription yield and the integrity of synthesized RNA. The greatest transcription yields are achieved with high purity plasmid templates. Plasmids purified by common laboratory methods can be used if the DNA is free of contaminating RNases, SDS, EDTA, proteins, salts* and RNA. DNA should have a A_{260}/A_{280} ratio of 1.8-2.0. The GeneJET™ Plasmid Miniprep Kit (#K0502) generates high purity plasmid DNA suitable for transcription.

* T7 and SP6 RNA Polymerases are inhibited by ~50% at NaCl or KCl concentrations above 150 mM and T3 RNA Polymerase – at above 250 mM.

Linearization

To produce RNA transcripts of a defined length, plasmid DNA is linearized by restriction digestion downstream of the insert. Restriction enzymes which generate blunt ends or 5'-overhangs are preferred. 3'-overhangs have been reported to generate spurious transcripts (1) and should therefore be avoided. 3'-overhangs can be blunted by T4 DNA Polymerase (#EPO061) prior to transcription.

Due to the high processivity of RNA polymerases, circular plasmid templates generate long heterogeneous RNA transcripts in higher quantities than linear templates. Therefore, it is important to completely linearize plasmid DNA to ensure efficient synthesis of defined length transcripts. See also "Troubleshooting Guide for DNA Digestion" on p.231. If complete digestion is unachievable, gel purify the linearized DNA

template prior to transcription reactions. After linearization, it is recommended to purify the DNA template using GeneJET™ PCR Purification Kit (#K0701) or by phenol/chloroform extraction:

1. Add 1/10 volume of 3 M Sodium Acetate Solution (#R1181) to the DNA.
2. Mix thoroughly.
3. Extract with an equal volume of a 1:1 phenol/chloroform mixture, and then twice with an equal volume of chloroform. Collect the aqueous phase and transfer to a new tube.
4. Precipitate the DNA by adding 2 volumes of ethanol. Incubate at -20°C for at least 30 min and collect the pellet by centrifugation.
5. Remove the supernatant and rinse the pellet with 500 µl of cold 70% ethanol.
6. Resuspend the DNA in 20 µl of DEPC-treated water (#R0601).

PCR Templates

PCR products can serve as templates for *in vitro* transcription. The RNA polymerase promoter must be located upstream of the sequence to be transcribed.

See "PCR, qPCR, RT-PCR & dNTPs" chapter or visit www.fermentas.com for information on Fermentas PCR kits and reagents.

6.2. Avoiding RNase contamination

Reagents supplied by Fermentas have been tested to ensure they are endo-, exodeoxyribonuclease, ribonuclease, and phosphatase free. However, an RNase-free working environment and RNase-free solutions are also critical factors for performing successful *in vitro* transcription.

General recommendations to avoid RNase contamination:

- Maintain a separate area, dedicated pipettors and reagents when working with RNA.
- Wear gloves when handling RNA and reagents to avoid contact with skin, which is a source of RNases. Change gloves frequently.
- Use sterile, RNase-free plastic tubes.
- Treat water and all solutions used for RNA purification and handling with DEPC. Add DEPC to 0.1% (v/v) final concentration; incubate overnight at room temperature and autoclave.
- High quality reagents must be used for buffer solutions. Buffers containing Tris should be prepared by dissolving Tris base in DEPC-treated water. Solutions containing DTT or nucleotides should be prepared using DEPC-treated water and be passed through a 0.2 µm filter for sterilization.
- Keep all kit components sealed when not in use and all tubes tightly closed during the transcription reaction.

6.3. *In vitro* transcription

More than 10 µg of RNA transcript can be generated per 1 µg template DNA using the following protocol. The reaction can be scaled up or down. For high yield transcription, generating up to 200 µg RNA, use TranscriptAid™ T7 High Yield Transcription Kit (#K0441).

- Thaw frozen reagents, mix and centrifuge briefly.
- Keep enzymes and nucleotides on ice.
- Keep the Reaction Buffer at room temperature.

1. Prepare the following reaction mixture at room temperature in the order given:

5X Transcription buffer	10 µl
ATP/GTP/CTP/UTP Mix, 10 mM each	10 µl (2 mM final concentration)
Linearized template DNA	1 µg
RiboLock™ RNase Inhibitor (#E00381)	1.25 µl (50 u)
T7/T3/SP6 RNA Polymerase (#EPO111, #EPO101, #EPO131)	1.5 µl (30 u)
DEPC-treated Water (#R0601)	to 50 µl
Total volume	50 µl

2. Incubate at 37°C for 2 hours.
3. Optional: To remove template DNA add 2 µl (2 u) of DNase I, RNase-free (#E0521), mix and incubate at 37°C for 15 min.
4. Stop the reaction by addition of 2 µl 0.5 M EDTA, pH 8.0 (#R1021) and incubate at 65°C for 10 min.

Note

RNA hydrolyzes if heated in the absence of a chelating agent.

6.4. Synthesis of radiolabeled RNA probes of high specific activity

1. Linearize template DNA with a restriction enzyme. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in DEPC-treated Water (#R0601).
2. Combine the following reaction components at room temperature in the order given:

5X Transcription buffer	4 µl
3 NTP Mix, 10 mM each* (without labeled NTP)	1 µl (0.5 mM final concentration)
100 µM CTP (#R0451)	2.4 µl (12 µM final concentration)
[α-³²P]-CTP, ~30 TBq/mmol (800 Ci/mmol)	1.85 MBq (50 µCi)
Linear template DNA	0.2-1.0 µg
RiboLock™ RNase Inhibitor (#E00381)	0.4 µl (20 u)
T7/T3/SP6 RNA Polymerase (#EPO111, #EPO101, #EPO131)	1 µl (20 u)
DEPC-treated Water (#R0601)	to 20 µl
Total volume	20 µl

3. Incubate at 37°C for 2 hours.
4. Stop the reaction by cooling at -20°C.
5. Determine the percentage of the label incorporated into RNA.

For patent and license information see p.557

**Note**

* To prepare a mix of the three non-labeled NTPs (10 mM) each, combine 1 µl of all three NTPs, 100 mM, from the set (#R0481) with 7 µl of DEPC-treated Water (#R0601). Store the mix at -20°C for further use.

- Expect specific radioactivity of 3-5 x10⁸ dpm/µg RNA.
- RNA can be radiolabeled with [³²P], [³⁵S] or [³H]-ribonucleotides. Recommended amounts of radiolabeled nucleotides in a 20 µl of reaction mixture are as follows:

Radiolabeled nucleoside	Amounts for 20 µl of reaction mixture	Specific activity
5'-[α- ³⁵ S]-UTP	11.1 MBq (300 µCi)	>37 TBq/mmol (1000 Ci/mmol)
5,6-[³ H]-UTP	0.925 MBq (25 µCi)	1.1-2.2 TBq/mmol (30-60 Ci/mmol)
5'-[α- ³² P]-CTP	1.85 MBq (50 µCi)	~30 TBq/mmol (800 Ci/mmol)

6.5. Purification of RNA transcripts**Template DNA**

May interfere with downstream applications of the RNA transcript. Template DNA should be removed by DNase I digestion directly after the transcription reaction. Add 2 u of DNase I, RNase-free (#EN0521), mix and incubate at 37°C for 15 min, then add 2 µl of 0.5 M EDTA, pH 8.0 (#R1021) and incubate at 65°C for 10 min to stop the reaction.

Proteins and nucleotides

Phenol/chloroform extraction and ethanol precipitation of RNA transcripts is recommended.

1. To a 50 µl reaction mixture, add 85 µl of DEPC-treated water (#R0601) and 15 µl of 3 M Sodium Acetate (#R1181). Mix thoroughly.
2. Extract with an equal volume of 1:1 phenol/chloroform mixture, and then twice with an equal volume of chloroform. Collect the aqueous phase and transfer to a new tube.
3. Precipitate the RNA by adding 2 volumes of ethanol. Incubate at -20°C for at least 30 min and collect the pellet by centrifugation.
4. Remove the supernatant and rinse the pellet with 500 µl of cold 70% ethanol.
5. Resuspend the RNA in 20 µl of DEPC-treated water (#R0601).
6. Store the RNA at -20°C or -70°C.

6.6. Evaluation of transcription reaction products**Quantification by UV Light Absorbance**

The easiest way to determine RNA concentration is to measure UV absorbance at 260 nm. Dilute an aliquot of the conventional transcription reaction 1:20 (1:300 for high yield transcription) to obtain an absorbance reading in the linear range of a spectrophotometer.

For single-stranded RNA, when $A_{260} = 1$, RNA concentration is 40 µg/ml. The RNA yield can be calculated as follows:

$$A_{260} \times (\text{dilution factor}) \times 40 = \mu\text{g/ml RNA.}$$

Note

Unincorporated nucleotides and template DNA in the mixture will interfere with quantification. Therefore, it is advisable to remove template and nucleotides from transcription mixture (see "Purification of RNA Transcripts" above).

Sizing and Quantification by Gel Electrophoresis

To evaluate transcript length, integrity and quantity, an aliquot of the transcription reaction should be run on a native or denaturing agarose gel or polyacrylamide gel along with the appropriate RNA ladder, e.g., RiboRuler™ High Range (#SM1821) or RiboRuler™ Low Range RNA Ladder (#SM1831).

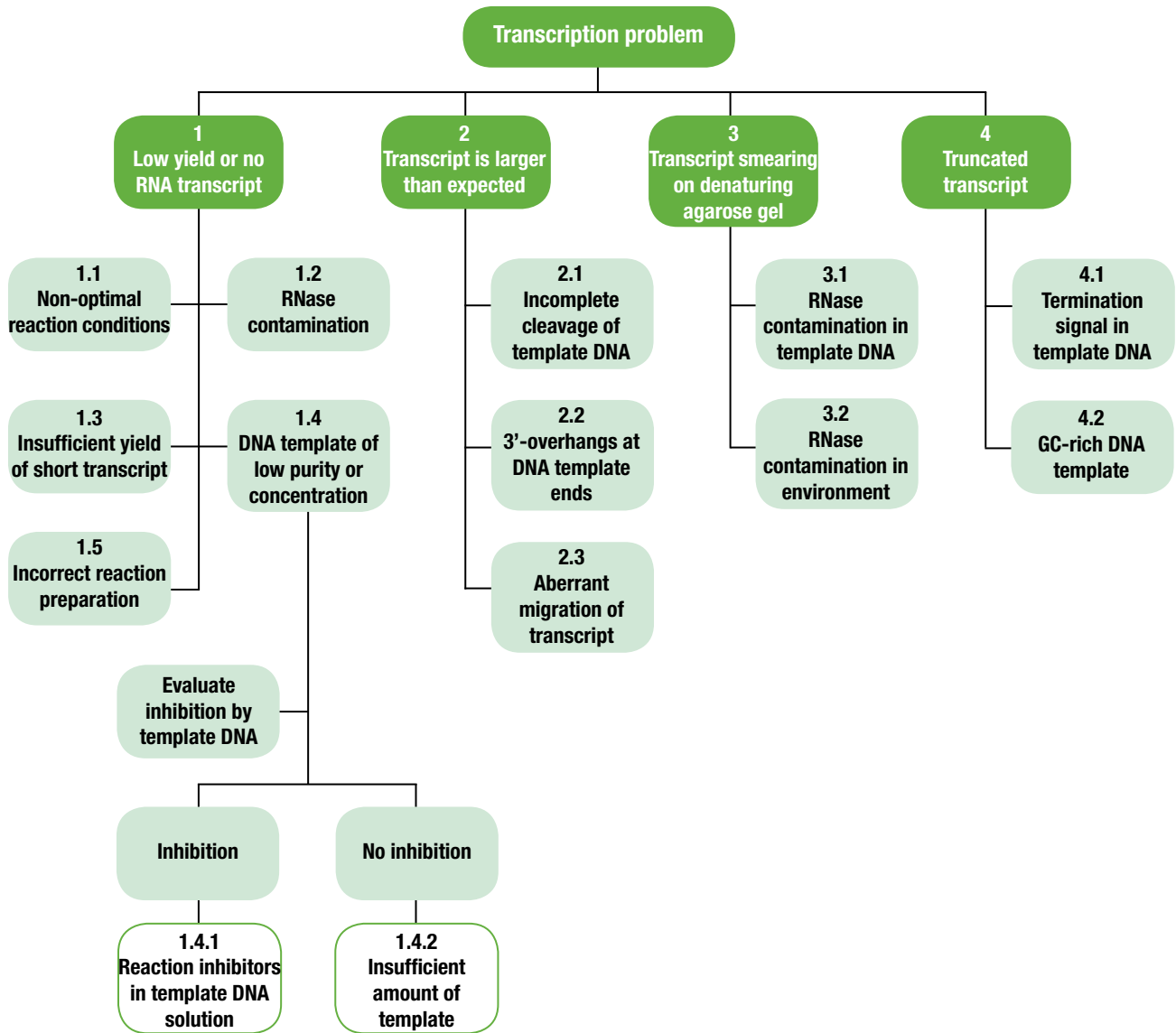
Transcript length	Recommended gel
>500 bases	1% agarose gel
100-500 bases	2% agarose gel or 4-5% denaturing polyacrylamide gel
50-100 bases	10% denaturing polyacrylamide gel or 2-3% agarose gel
<50 bases	20% denaturing polyacrylamide gel or 3-4% agarose gel

Recommendations for RNA Sample Loading

- Use only fresh electrophoresis buffers and freshly poured gels.
- Use clean electrophoresis chambers. For RNA gel analysis, avoid electrophoresis tanks used for DNA miniprep analysis since DNA minipreps often contain RNase A or T1.
- Use the RiboRuler™ High Range or RiboRuler™ Low Range RNA Ladder (#SM1831, #SM1821) for sizing and approximate quantification of the transcript.
- Use the same loading dye for samples and for RNA ladders. 2X RNA Loading Dye (#R0641) is available separately and is provided with all Fermentas RiboRuler™ RNA Ladders. The loading dye contains ethidium bromide for RNA visualization on denaturing formaldehyde gels.
- For native gels, add 0.5 µg/ml of ethidium bromide to the agarose gel and to the running buffer.

1. Dilute the RNA transcript with DEPC-treated water (#R0601) to a final concentration of 0.1-0.5 µg/µl.
2. Mix 2-4 µl (0.5-1 µg RNA) of diluted sample with an equal volume of 2X RNA Loading Dye (#R0641). If using a non ready-to-use version of the RNA ladder, mix it with the loading dye solution as well. Use 0.25 µl of conventional ladder per 1 mm of the gel lane width.
3. Heat samples and ladder for 10 min at 70°C.
4. Chill samples and ladder on ice for 3 min and spin briefly prior to loading.
5. Load 1 µl of sample per 1 mm of gel lane width.
6. Run the RNA ladder in parallel with your samples. Use 0.5 µl of the ready-to-use ladder per 1 mm of gel lane width.
7. Run the gel at 5 V/cm.

Troubleshooting Guide



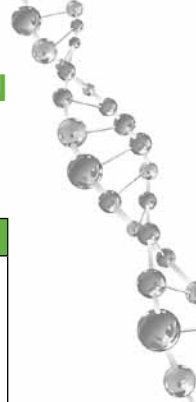


Table 6.1. Troubleshooting guide for *in vitro* transcription.

Problem	Possible cause and recommended solution								
1. Low yield or no RNA transcript	<p>1.1. Non-optimal reaction conditions. A conventional <i>in vitro</i> transcription reaction, using stand alone RNA polymerases should produce at least 10 µg of RNA transcript from 1 µg of template. For higher RNA yields (up to 200 µg), the TranscriptAid™ T7 High Yield Transcription Kit (#K0441) should be used. Addition of Pyrophosphatase, Inorganic (#EF0221) at 0.02-0.1 u (0.2-1 µl) per 20 µl of reaction volume may increase the yield of RNA by reducing the effect of reaction inhibition by pyrophosphates.</p>								
	<p>1.2. RNase contamination. Working environment, DNA template, reagents or electrophoresis systems may be contaminated with RNases.</p> <ul style="list-style-type: none"> • Follow general recommendations for working with RNA (p.368). • Use RNase-free enzymes, nucleotides and DEPC-treated water (#R0601). • Use RiboLock™ RNase Inhibitor (#E00381) to protect synthesized RNA from RNases. <p>Note RiboLock™ RNase Inhibitor inhibits the activity of RNases A, B and C. It does not inhibit the following RNases: I, T1, T2, H, U1, U2 and CL3. Do not use electrophoresis tanks which have been previously used for plasmid DNA miniprep analysis as they may be contaminated with RNases A or T1.</p>								
	<p>1.3. Insufficient yield of short transcript. High yields of short transcripts (≤100 bases) can be achieved by increasing the amount of template and extending the incubation time. Use 2 µg of template and prolong the reaction time to 4-8 hours.</p>								
	<p>1.4. DNA template of low purity or concentration. Evaluate your template in conjunction with a control template to determine if contaminants are inhibiting the reaction. If your template generates considerably lower RNA yields compared to the control template, modify the transcription reaction described on p.368 by mixing equal amounts of experimental template to the control template and adjusting the volume of DEPC-treated water (#R0601). Evaluate the transcript on an agarose gel as described on p.369:</p> <div style="text-align: center; margin: 10px 0;"> <table border="1" style="border-collapse: collapse; width: 100%;"> <thead> <tr> <th style="padding: 2px 5px;">C</th> <th style="padding: 2px 5px;">S</th> <th style="padding: 2px 5px;">C/S1</th> <th style="padding: 2px 5px;">C/S2</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; padding: 5px;"></td> <td style="text-align: center; padding: 5px;"></td> <td style="text-align: center; padding: 5px;"></td> <td style="text-align: center; padding: 5px;"></td> </tr> </tbody> </table> </div> <p>Figure 6.4. Evaluation of mixing experiment results C – control template S – sample template C/S1 – mixture of C and S: control reaction inhibited by sample template solution C/S2 – mixture of C and S: control reaction not inhibited by sample template</p> <p>If control reaction was inhibited by sample template (see Fig. 6.4 C/S1), this indicates:</p> <p>1.4.1. Reaction inhibitors in template DNA solution. Template DNA may contain residual SDS, EDTA, proteins, salts* and RNases. Repurify the template by phenol/chloroform extraction and ethanol precipitation. An A₂₆₀/A₂₈₀ ratio of 1.8-2.0 should be observed. To remove EDTA and salts, wash the pellet with 70% cold ethanol. * T7 and SP6 RNA Polymerases show ~50% inhibition by NaCl or KCl at concentrations above 150 mM; T3 RNA Polymerase at above 250 mM. Greater than 50% inhibition of the polymerases is observed with ammonium sulphate.</p> <p>If the control reaction is not inhibited by the sample template (see Fig. 6.4 C/S2), but low RNA yields are observed, this indicates:</p> <p>1.4.2. Insufficient amount of template. Low amounts of template produce significantly lower RNA yields. The presence of RNA and chromosomal DNA in the DNA template preparation may interfere with UV absorbance readings and lead to misinterpretation of template DNA concentration. To accurately determine the concentration, size and integrity of the template, analyze the DNA concentration by UV absorbance and gel electrophoresis.</p>	C	S	C/S1	C/S2				
	C	S	C/S1	C/S2					
<p>1.5. Incorrect reaction preparation. If the reaction is prepared on ice or in the incorrect order, the DNA may precipitate in the presence of spermidine in the reaction buffer. Water should always be added to the transcription reaction first.</p>									

(continued on next page)

Table 6.1. Troubleshooting guide for *in vitro* transcription.

Problem	Possible cause and recommended solution
2. Transcript is larger than expected	2.1. Incomplete cleavage of template plasmid DNA. Even small amounts of undigested circular DNA can produce large amounts of long transcripts. Check the template for complete digestion and, if required, additionally digest with the appropriate restriction enzyme (see Troubleshooting Guide for DNA Digestion on p.231). For faster and more efficient plasmid cleavage, use FastDigest® enzymes. If complete digestion is unachievable, gel-purify the digested template.
	2.2. 3'-overhangs at DNA template ends. Avoid plasmid linearization with restriction enzymes that generate 3'-overhangs. Alternatively, blunt 3'-overhangs with T4 DNA Polymerase (#EP0061) before use in transcription. See protocol on p.338.
	2.3. Aberrant migration of transcript. Due to secondary structures, RNA may run aberrantly on a native gel. On a denaturing gel, these transcripts normally migrate as single bands at the expected size.
3. Transcript smearing on denaturing agarose gel	3.1. RNase contamination in template DNA. During preparation, plasmid DNA templates are often contaminated with RNases. This can affect the length and yield of synthesized RNA, and is seen as a smear below the expected transcript length. If using a commercial kit, such as the GeneJET™ Plasmid Miniprep Kit (#K0502), omit the RNase A from plasmid preparation solutions and use DEPC-treated water (#R0601) for plasmid elution. If RNase A is pre-mixed in the purification buffers, perform phenol/chloroform extraction after plasmid DNA linearization, then ethanol precipitate the DNA and dissolve in DEPC-treated water (#R0601) (see Plasmid Templates on p.368).
	3.2. RNase contamination in working environment. <ul style="list-style-type: none"> Follow general recommendations for working with RNA (p.368). Use RNase-free enzymes, nucleotides and water. Use RiboLock™ RNase Inhibitor (#E00381) to protect synthesized RNA from RNases*. Do not use electrophoresis tanks which have been previously used for plasmid DNA miniprep analysis as they may be contaminated with RNases A or T1. <small>* RiboLock™ RNase Inhibitor inhibits the activity of RNases A, B and C. It does not inhibit the following RNases: I, T1, T2, H, U1, U2 and CL3.</small>
4. Truncated transcript	4.1. RNA polymerase recognizes a termination signal in template DNA sequence. Try another RNA polymerase system or perform the transcription reaction at a lower temperature (e.g. 30°C). This may increase the length of transcript. However, RNA yield may be decreased at lower temperatures.
	4.2. GC-rich DNA template (or template with high secondary structure). For templates with secondary structure, incubating at 42°C or using a single-stranded binding (SSB) protein has been reported to improve yield and transcript length (2).

References

- Schenborn, E.T and Mierendorf, R.C., Nucl. Acids Res.,13, 6223-6236, 1985.
- Aziz, R.B. and Soreq, H., Nucl. Acids Res., 18, 3418, 1990.