Manual

AmpliScribe T7-*Flash* Transcription Kit

For Research Use Only. Not for use in diagnostic procedures.

AmpliScribe™ T7-*Flash*™ Transcription Kit is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Manual

AmpliScribe T7-*Flash* Transcription Kit

Contents

Manual

AmpliScribe T7-*Flash* Transcription Kit

1. Introduction

The AmpliScribe T7-Flash Transcription Kit is specially formulated to enable users to obtain the highest yields of RNA from an in vitro transcription reaction in just 30 minutes. A standard 30 minute, 20 µL AmpliScribe T7-Flash reaction will produce 160-180 µg of RNA from 1 µg of the linearised control DNA template.

The AmpliScribe T7-Flash Transcription Kit can be used to produce RNA transcripts of a wide range of sizes (from <50 b to >9 kb). Standard AmpliScribe T7-Flash reactions can also be scaled up to produce milligram amounts of RNA in a single reaction.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
AmpliScribe T7-*Flash* Transcription Kit	25 reactions	ASF3257	T7 RNA Polymerase	E0005NI-D3	50 µL
			10X Transcription Reaction Buffer	SS000651-D4	125 µL
			ATP (100 mM)	SS000210-D1	50 µL
			GTP (100 mM)	SS000207-D1	50 µL
			CTP (100 mM)	SS000208-D1	50 µL
			UTP (100 mM)	SS000209-D1	50 µL
			DTT (100 mM)	SS000065-D3	50 µL
			RiboGuard RNase Inhibitor (40 U/µL)	E0126-40D2	15 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1.0 mL
			Control Template DNA (0.5 µg/µL)	SS000571-D1	10 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D3	25 µL
	50 reactions	ASF3507	T7 RNA Polymerase	E0005NI-D6	120 µL
			10X Transcription Reaction Buffer	SS000651-D5	250 µL
			ATP (100 mM)	SS000210-D3	100 µL
			GTP (100 mM)	SS000207-D3	100 µL
			CTP (100 mM)	SS000208-D3	100 µL
			UTP (100 mM)	SS000209-D3	100 µL
			DTT (100 mM)	SS000065-D5	125 µL
			RiboGuard RNase Inhibitor (40 U/µL)	E0126-40D5	30 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1.0 mL
			Control Template DNA (0.5 µg/µL)	SS000571-D2	25 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D7	60 µL

3. Product specifications

Storage: Store only at -20 °C in a freezer without a defrost cycle. Do not store at -70 °C.

Contaminating activity assays: All of the components of the AmpliScribe T7-*Flash* Transcription Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

Control template: The control template is a 4.2-kb linearised plasmid, containing a 1.4-kb lambda DNA insert, that will produce a 1,380-b runoff transcript.

DNase I unit definition: 1 Molecular Biology Unit (MBU) of DNase I digests 1 microgram of pUC19 DNA to oligodeoxynucleotides in 10 minutes at 37 °C.

4. Notes on using the AmpliScribe T7-*Flash* Transcription Kit

1. **Template preparation:** Transcription templates should be linear double-stranded DNA with blunt or 5′-protruding ends. Templates containing 3′-protruding ends can produce spurious transcripts due to non-specific initiation. PCR products and cDNA can also be used as templates, provided that the appropriate promoter has been incorporated into one of the primers used.

   The quality of the DNA template directly affects the quantity and quality of the RNA produced. Generally, DNA is of sufficient quality for use if it is free of contaminating RNase and can be fully digested with restriction enzymes. To confirm that a template is fully linearized and intact, examine the DNA on an ethidium-stained agarose or polyacrylamide gel prior to use.

   Templates that give low yields or less than full-length transcripts may contain RNase or other contaminants. Such templates will usually give better results after the following treatment:

   a) Add Proteinase K to 100-200 µg/mL and SDS to 0.5%.
   b) Incubate for 30-60 minutes at 37 °C.
   c) Extract with an equal volume of a 1:1 mixture of TE-saturated phenol/chloroform.
   d) Ethanol precipitate.
   e) Gently remove the supernatant and rinse the pellet with 70% ethanol.
   f) Resuspend at 1.0 µg/µL in RNase-Free TE (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

2. **Template efficiency:** Linearised plasmid templates and PCR product templates which produce transcripts of equivalent sizes are utilised with equal efficiency by the AmpliScribe T7-*Flash* Transcription Kit. The Control Template DNA produces 160-180 µg of a ~1.4-kb RNA per 1 µg of DNA template in a standard 20-µL, AmpliScribe T7-*Flash* reaction. Different templates may give different yields. Lower yields from an experimental template could be due to:

   a) *Quality of template prep:* Degraded templates, RNase or contaminants such as phenol, trace metals and SDS may reduce yields.
   b) *Transcriptional efficiency:* Different templates may be transcribed more or less efficiently based on promoter strength, reinitiation rate and termination efficiency.
   c) *Size of the template:* Yields may also differ based on the size of the template. For example, in 30 minutes, 1 µg of template DNA (ranging in size from 1-9 kb) can produce 150-210 µg of RNA, while 1 µg of template DNA (ranging in size from 26- 335 b) can produce 12-76 µg of RNA.

3. **Amount of template:** The standard 20-µL, 30-minute AmpliScribe T7-*Flash* reaction was optimised for transcription using 1 µg of linear DNA template, however, higher or lower amounts of DNA template can be used successfully in an AmpliScribe T7-*Flash* reaction. Table 1 summarises our experiences with varying the amount of control DNA template in a standard AmpliScribe T7-*Flash* reaction. Results may vary depending on the template used. Increasing the reaction time for lower amounts of template **may** increase the yield of RNA. Reactions containing higher amounts of template **may not** require a full 30-minute incubation.

Template DNA (µg)	Incubation time (minutes)					
	10	15	20	30	60	120
0.10	---	---	---	---	79 µg	134 µg
0.25	---	---	---	68 µg	112 µg	168 µg
0.50	---	---	---	124 µg	176 µg	164 µg
0.75	---	---	116 µg	156 µg	168 µg	180 µg
1.0	---	108 µg	140 µg	172 µg	168 µg	176 µg
2.0	108 µg	156 µg	164 µg	172 µg	172 µg	172 µg
3.0	136 µg	160 µg	176 µg	170 µg	180 µg	176 µg

Table 1. Yield of RNA (in µg) from varying amounts of Control Template DNA from a standard 37 °C, 20-µL AmpliScribe T7-*Flash* Reaction over time. Results may vary depending on the template used.

4. **Reaction assembly: assemble an AmpliScribe T7-*Flash* transcription reaction at room temperature!** Assembly of the reaction at temperatures less than 22 °C can result in formation of an insoluble precipitate. Storing the 10X Transcription Reaction Buffer at -70 °C may result in the formation of a white precipitate. If this happens, heat the tube to 37 °C for 5 minutes and mix thoroughly to resuspend the precipitate.
5. **Optimising the reaction:** The recommended reaction conditions should give excellent results with most templates. Modifying the protocol may, however, improve results with some templates. One way to increase yield is to extend the incubation time. With the Control Template DNA, incubation for an additional 30 minutes can increase yields 10-15%. A second way to increase yield in some cases is to raise the template concentration (see Table 1). Finally, increasing the reaction temperature from 37 °C to 42 °C may often improve the yield.
6. **Yield of "short" (<1 kb) RNA transcripts:** Although the number of micrograms of short RNA produced in a standard AmpliScribe T7-*Flash* reaction is small compared to the yield of "long" (>1 kb) transcripts, the number of **moles** of short RNA produced is most often greater than the number of **moles** of long RNA produced (e.g., 200 µg of a 7-kb RNA is 0.09 nmol). Yields of short RNA can be increased by:
   a) increasing the amount of DNA template used in the reaction.
   b) increasing the reaction time.
   c) increasing the reaction temperature from 37 °C to 42 °C.

Runoff template DNA size (bases)	Incubation time (minutes) 10	15	20	30	60
26 b	20 µg 2.33 nmol	36 µg 4.20 nmol	56 µg 6.53 nmol	88 µg 10.26 nmol	104 µg 12.12 nmol
47 b	24 µg 1.55 nmol	36 µg 2.32 nmol	62 µg 4.00 nmol	88 µg 5.67 nmol	104 µg 6.70 nmol
96 b	36 µg 1.14 nmol	60 µg 1.89 nmol	92 µg 2.90 nmol	128 µg 4.04 nmol	144 µg 4.55 nmol
335 b	76 µg 0.69 nmol	120 µg 1.09 nmol	152 µg 1.38 nmol	---	---

Table 2. Yield of short RNA (in µg and nmol) for short template DNAs from a standard 37 °C, 20-µL AmpliScribe™ T7-*Flash* Reaction with varying times of incubation. Results may vary depending on the template used.

5. Standard AmpliScribe T7-*Flash* Transcription Reaction

1. ***Important!* Combine the following reaction components at room temperature in the order given.** (see Note 4)

x	µL Nuclease-Free Water, Sterile
1	µg linearised template DNA with appropriate promoter*
2	µL 10X Transcription Reaction Buffer
1.8	µL 100 mM ATP
1.8	µL 100 mM CTP
1.8	µL 100 mM GTP
1.8	µL 100 mM UTP
2	µL 100 mM DTT
0.5	µL RiboGuard RNase Inhibitor
2	µL T7 RNA Polymerase
20	µL Total reaction volume

2. Incubate at 37 °C for 30 minutes.
   ***Note: I**ncubating the reaction at 42 °C may increase yields by about 10%.*
   Optional: Treat the sample with RNase-Free DNase I to remove DNA template and purify the RNA as described in “Purification of the RNA”.
   **More or less DNA template can be added to the reaction. See Notes 3 and 6 for further information.*

6. Scale-up an AmpliScribe T7-*Flash* Transcription reaction

AmpliScribe T7-*Flash* reactions can be scaled-up by two different methods, to produce milligram amounts of RNA in a single reaction tube.

Method 1 Scale-up all reaction components proportionally, *including the template DNA*.
This method minimises the reaction time required for a completed reaction but requires more DNA template. For example, 1 mg of a 1.4 kb transcript can be produced in 30 minutes from the Control Template DNA by a 6X scale-up of the standard 20 µL reaction to 120 µL using 6 µg of Control Template DNA.

Method 2 Scale-up all reaction components proportionally, *except the template DNA*.
This method minimises the amount of DNA template required for a completed reaction but requires longer reaction times. For example, 1 mg of a 1.4 kb transcript can be produced in 120 minutes from the Control Template DNA by an 8X scale-up of the standard 20 µL reaction to 160 µL using 1 µg of Control Template DNA.

1. ***Important!* Combine the following reaction components at room temperature in the order given** (see Note 4). Using the 'Standard AmpliScribe T7-*Flash* Transcription reaction' (see above) as a guide, combine and mix the appropriate volume of each reaction component.
2. Incubate the reaction for 30 minutes (for Method 1) or longer (for Method 2) at 37 °C.
   Note: *Incubating the reaction at 42 °C may increase yields by about 10%.*
3. Optional: Treat the sample with a proportionally scaled-up volume of RNase-Free DNase I to remove the DNA template (below). Purify the RNA as described in "Purification of the RNA".

7. DNase I treatment

If removal of the DNA template is desired, digest with RNase-Free DNase I.

1. Add 1 µL (1 MBU) of RNase-Free DNase I to the standard 20 µL AmpliScribe T7-*Flash* Transcription reaction and incubate for 15 minutes at 37 °C.
2. Extract with TE-saturated phenol/chloroform, followed by extraction with chloroform. Ethanol precipitate the RNA or precipitate the RNA using ammonium acetate as described in "Purification of the RNA".

8. Purification of the RNA

For RNA transcripts >100 bases, the RNA can be purified by ammonium acetate precipitation.
This method selectively precipitates RNA while leaving much (but not all) of the DNA, protein and unincorporated NTPs in the supernatant.

1. Add 1 volume of 5 M ammonium acetate (20 µL for the standard AmpliScribe T7-*Flash* reaction).
2. Incubate on ice for 10-15 minutes.
3. Centrifuge at high speed (e.g., 10,000 x g) for 10-15 minutes at room temperature or 4 °C.
4. Wash the pellet in 70% ethanol.
5. RNA can be stored at -20 °C or -70 °C as a dry pellet or resuspended in Nuclease-Free Water, Sterile; TE; or other suitable buffer.

For RNA transcripts <100 bases, remove the unincorporated NTPs by chromatography followed by ethanol precipitation.

1. Remove unincorporated NTPs by spin column chromatography. For commercially- available columns, follow the manufacturer's instructions for this step.
2. Add sodium acetate to 0.3 M, followed by 2.5 volumes of ethanol.
3. Incubate at -20 °C for 30 minutes and collect by centrifugation.
4. Remove the supernatant carefully with a pipette and gently rinse the pellet with 70% ethanol.
5. RNA can be stored at -20 °C or -70 °C as an ethanol pellet or resuspended in Nuclease- Free Water, Sterile; TE; or other suitable buffer.

9. Analysis of the RNA transcript

Use a denaturing agarose gel1 of appropriate concentration for the RNA transcription product.

Denaturing gels allow in vitro transcripts to separate on the basis of their length rather than based on their length plus secondary structure. Denaturing conditions for electrophoresis will remove any secondary structures from the RNA and allow the RNA to migrate in a tight band rather than a smear which can occur with native gels.

10. Reference

1. Molecular Cloning - A Laboratory Manual, Third Edition, 2001. CSHL Press. pp 7.27-7.34. J. Sambrook and D. Russell.

11. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

@LGCBiosearch | biosearchtech.com

GEN/521/EK/1122

For Research Use Only. Not for use in diagnostic procedures.

bsi. ISO 13485 Medical Devices Quality Management
FM 598408

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC