Manual

AmpliScribe T7-*Flash* Biotin-RNA Transcription Kit

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AmpliScribe™ T7-*Flash*™ Biotin-RNA 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.

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GENOMIC ANALYSIS BY LGC

Manual

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1. Introduction

The AmpliScribe T7-*Flash* Biotin-RNA Transcription Kit utilises the LCG Biosearch Technologies™ high yield AmpliScribe *Flash in vitro* transcription technology and Biotin-16-UTP (provided in the kit) to produce high yields of biotin-labelled RNA. Linearised plasmids, double-stranded cDNA and PCR products containing a phage T7 transcription promoter are efficiently transcribed. An AmpliScribe T7-*Flash* Biotin-RNA Transcription Kit reaction can be performed using 50 ng to1 µg of input Control Template DNA. The *in vitro* transcription reaction conditions have been optimised to produce the highest yield of biotin-RNA with high incorporation of biotin-UTP.

Applications

1. Production of non-radioactive RNA probes for:
   - DNA microarray experiments
   - *In situ* hybridisation experiments
   - Blotting experiments
2. Subtractive hybridisation studies

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
AmpliScribe T7-*Flash* Biotin-RNA Transcription Kit	10 rxns	ASB71110	T7 RNA Polymerase	E0005NI-D1	25 µL
			10X Transcription Reaction Buffer	SS000651-D2	40 µL
			NTP/Biotin-UTP PreMix	SS000801-D	90 µL
			DTT (100 mM)	SS000065-D2	25 µL
			RiboGuard RNase Inhibitor (40 U/µL)	E0126-40D2	15 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1 mL
			Control Template DNA (0.5 µg/µL)	SS000571-D1	10 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D3	25 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* Biotin-RNA Transcription Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

The control template: The Control Template DNA 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 RNase-Free DNase I digests 1 µg of pUC19 DNA to oligodeoxynucleotides in 10 minutes at 37 °C.

4. Notes on using the AmpliScribe T7-*Flash* Biotin-RNA 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 (e.g. double-stranded cDNA template produced during an "Eberwine" RNA amplification reaction) 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 linearised 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:** The Control Template DNA produces greater than 180 µg of ~1.4 kb biotin-RNA per 1 µg of DNA template in a 60 minute 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.

3. **RNA yield, amount of plasmid DNA template and reaction time:** The standard reaction produces exceptionally high yields of biotin-RNA using 1 µg of linearised plasmid DNA template in 60 minutes. However, higher or lower amounts of DNA template can be used successfully. Table 1 summarises our experiences with varying the amount of control DNA template in a standard AmpliScribe T7-*Flash* Biotin-RNA Transcription reaction. Results may vary depending on the template used.

		Incubation time (minutes)			
		30	60	120	240
Control template DNA	50 ng	5-10 µg	15-25 µg	30-40 µg	60-70 µg
	100 ng	15-20 µg	35-45 µg	70-80 µg	130-140 µg
	500 ng	60-70 µg	140-150 µg	>180 µg	>180 µg
	1.0 µg	125-135 µg	>180 µg	>180 µg	>180 µg

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

4. **Reaction assembly:** Assemble an AmpliScribe T7-*Flash* Biotin-RNA 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 buffer to 37 °C for 5 minutes and mix thoroughly to dissolve the precipitate.
5. **Maintaining an RNase-free environment:** The RiboGuard RNase Inhibitor included in the kit is a potent RNase inhibitor. However, creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful *in vitro* transcription reactions. Therefore, we strongly recommend the user:
   a) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils and human skin.
   b) Always wear gloves when handling kit components. Do not pick up any kit component with an ungloved hand.
   c) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

Standard AmpliScribe T7-*Flash* Biotin-RNA Transcription reaction

We recommend that you perform all reactions in sterile (RNase-free) tubes using sterile pipette tips and recently calibrated pipettors. Wear gloves when handling all kit components and reaction tubes.

1. Place the T7 RNA Polymerase on ice.
   Thaw the remaining *in vitro* transcription reagents at room temperature.
2. Thoroughly mix the thawed 10X Transcription Reaction Buffer before use.
   IMPORTANT! *If a precipitate is visible in the thawed 10X Transcription Reaction Buffer, heat the buffer to 37 °C until it dissolves. Mix the buffer thoroughly. Keep the buffer at room temperature.*
3. Combine the following reaction components at room temperature in the order given.

x	µL	Nuclease-Free Water, Sterile
50 ng - 1	µg	linearised template DNA
2	µL	10X Transcription Reaction Buffer
8	µL	NTP/Biotin-UTP PreMix
2	µL	100 mM DTT
0.5	µL	RiboGuard RNase Inhibitor
2	µL	T7 RNA Polymerase
20	µL	Total reaction volume

4. Incubate at 37 °C.
   Refer to Table 1 to determine the reaction time that will maximise the yield of biotin-RNA from the amount of template used in the reaction.

5. Optional: If removal of the DNA template is desired, add 1 µL (1 MBU) of RNase-Free DNase I to the standard 20 µL reaction and incubate for 15 minutes at 37 °C.

Purifying the biotin-RNA

Biotin-RNA >100 bases can be purified by ammonium acetate precipitation (described below) or by spin column chromatography using commercially available columns. If purifying the biotin-RNA using spin columns, follow the manufacturer's protocol except, elute the biotin-RNA from the column using two elution steps.

Biotin-RNA <100 bases should be purified by spin column chromatography.

Elute the biotin-RNA from the columns using two elution steps.

Analysis of the RNA transcript

Use a denaturing agarose gel[1] 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.

Ammonium acetate precipitation of biotin-RNA

1. Add 1 volume of 5 M ammonium acetate (20 µL for the standard AmpliScribe T7-*Flash* Biotin-RNA Transcription 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. Biotin-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.

Quantifying the concentration and yield of the biotin-RNA

Concentration and yield: Due to the high yield of biotin-RNA that is produced, the yield and concentration of biotin-RNA can be determined easily and rapidly by UV spectroscopy.

1. Prepare a dilution of the biotin-RNA into the minimum volume of water or TE buffer required by the spectrophotometer cuvette that will be used.
2. Zero the spectrophotometer at 260 nm using the diluent (water or TE buffer) that was used to dilute the biotin-RNA sample.
3. Measure and record the absorbance of the diluted biotin-RNA at 260 nm (A_{260}).
4. Calculate the concentration of the biotin-RNA. Use the conversion factor that an A_{260} reading of 1.0 is equal to an RNA concentration of 40 µg/mL.
   Biotin-RNA concentration = (A_{260} reading) x (dilution factor) x (40 µg/mL).
   Example: Dilution for A_{260} measurement = 1:200 with an A_{260} of the 1:200 dilution = 0.20.
   Biotin-RNA concentration = (0.20) × (200) × (40 µg/mL) = 1,600 µg/mL = (1.6 µg/µL) biotin-RNA.

5. Calculate the yield of biotin-RNA using the formula:
   Yield of biotin-RNA = (biotin-RNA Concentration) x (Volume of biotin-RNA).
   Example: 100 µL of biotin-RNA recovered, 1.6 µg/µL biotin-RNA determined above.
   Biotin-RNA yield = (1.6 µg/µL) × (100 µL) = 160 µg of biotin-RNA.

5. Reference

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

6. Further support

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

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