

# AmpliScribe™ T7-Flash™ Biotin-RNA Transcription Kit

Cat. No. ASB71110

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## 1. Introduction

The AmpliScribe™ T7-Flash™ Biotin-RNA Transcription Kit utilizes Epicentre's high yield AmpliScribe *Flash in vitro* transcription technology and Biotin-16-UTP (provided in the kit) to produce high yields of biotin-labeled RNA. Linearized 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-1 µg of input control DNA template. The *in vitro* transcription reaction conditions have been optimized 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* hybridization experiments
  - Blotting experiments
2. Subtraction hybridization studies

## 2. 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.

**Control Template:** The control template is a 4.2-kb linearized 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.

## 3. Kit Contents

Desc.	Concentration	Quantity
<b>AmpliScribe™ T7-Flash™ Biotin-RNA Transcription Kit Contents</b>		
The AmpliScribe T7-Flash Biotin-RNA Transcription Kit contains sufficient reagents to perform 10 reactions.		
AmpliScribe™ T7-Flash™ RNA Polymerase		25 µl
NTP / Biotin-UTP PreMix		90 µl
AmpliScribe™ T7-Flash™		
10X Reaction Buffer		25 µl
100 mM Dithiothreitol (DTT)		25 µl
RiboGuard™ RNase Inhibitor		10 µl
RNase-Free Water		100 µl
Control Template DNA	@ 0.5 µg/µl	10 µl
RNase-Free DNase I	@ 1 MBU/µl	15 µl

Large volume discounts are available and the kit is available in bulk. Please inquire.

#### 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 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:

- Add Proteinase K to 100-200 µg/ml and SDS to 0.5%.
  - Incubate for 30-60 minutes at 37°C.
  - Extract with an equal volume of a 1:1 mixture of TE-saturated phenol/chloroform.
  - Ethanol precipitate.
  - Gently remove the supernatant and rinse the pellet with 70% ethanol.
  - Resuspend at 1.0 µg/µl in RNase-Free T<sub>10</sub>E<sub>1</sub> (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).
- 2. Template Efficiency:** The Control DNA Template 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:
    - Quality of template prep:* Degraded templates, RNase or contaminants such as phenol, trace metals, and SDS may reduce yields.
    - Transcriptional efficiency:* Different templates may be transcribed more or less efficiently based on promoter strength, reinitiation rate and termination efficiency.
    - 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 linearized plasmid DNA template in 60 minutes. However, higher or lower amounts of DNA template can be used successfully. Table 1 summarizes 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.

**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.

		Incubation Time (minutes)			
		30	60	120	240
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

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 AmpliScribe T7-Flash 10X 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 AmpliScribe T7-Flash RNA Polymerase on ice.  
Thaw the remaining *in vitro* transcription reagents at room temperature.
2. Thoroughly mix the thawed AmpliScribe T7-Flash 10X Reaction Buffer before use.

**Important!** If a precipitate is visible in the thawed AmpliScribe T7-Flash 10X 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	RNase-Free water
50 ng – 1	μg	linearized template DNA
2	μl	AmpliScribe T7-Flash 10X Reaction Buffer
8	μl	NTP / Biotin-UTP PreMix
2	μl	100 mM DTT
0.5	μl	RiboGuard RNase Inhibitor
2	μl	AmpliScribe T7-Flash Enzyme Solution
20	μl	Total reaction volume
4. Incubate at 37°C.  
Refer to Table 1 to determine the reaction time that will maximize 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 2 elution steps.

Biotin-RNA <100 bases should be purified by spin column chromatography. Elute the biotin-RNA from the columns using 2 elution steps.

### Ammonium Acetate Precipitation of Biotin-RNA

**Note:** 5M Ammonium acetate is available separately from Epicentre, see page 5.

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 RNase-Free water, T<sub>10</sub>E<sub>1</sub>, 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 diluents (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 =  $(0.20) \times (200) \times (40 \text{ µg/ml}) = 1,600 \text{ µg/ml} = (1.6 \text{ µg/µl})$  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 =  $(1.6 \text{ µg/µl}) \times (100 \text{ µl}) = 160 \text{ µg}$  of Biotin-RNA.

## 5. Related Products

The following products are also available:

### 5M Ammonium Acetate

G1005ML	5 ml
G1025ML	25 ml

Ammonium acetate precipitation can be used to purify Biotin-RNA >100 bases in size.

### TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104

TAB1R6910	10 Reactions
TAB1R6924	24 Reactions

Produces microgram amounts of Biotin-aRNA (also called cRNA) from as little as 25 ng of total RNA.

### TargetAmp™ Nano Labeling Kit for Illumina® Expression BeadChip®

TAN70924	24 Reactions
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Produces microgram amounts of Biotin-aRNA (also called cRNA) from as little as 25 ng of total RNA for use with Illumina® gene expression system such as the Illumina® Expression BeadChips.

### TargetAmp™ 2-Round Biotin-aRNA Amplification Kit 3.0

TAB2R91010	10 Reactions
TAB2R91024	24 Reactions

Produces microgram amounts of Biotin-aRNA (also called cRNA) from as little as 50 pg of total RNA.

**AmpliScribe™ T7-Flash™ Transcription Kit**

ASF3257 25 Reactions

ASF3507 50 Reactions

Produces the highest yields of unlabeled RNA from an *in vitro* transcription reaction using very short reaction times (e.g., >180 µg of RNA from 1 µg control DNA template in 30 minutes).

**Biotin-16-UTP Solution**

BU6105H 500 nmol @ 50 mM

Biotin-16-UTP is supplied in a convenient 50 mM solution.

**Biotin-X-X-NHS**

BXX51005 5 x 2.5 mg vials

BXX51010 10 x 2.5 mg vials

Biotin-X-X-NHS can be used to rapidly and cost-effectively label aminoallyl-aRNA and aminoallyl-DNA with biotin for use in DNA microarrays or other biotin-probe applications.

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