



# AmpliScribe™ T7-Flash™ Transcription Kit

Cat. Nos. ASF3257 and ASF3507

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## 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- $\mu$ l AmpliScribe T7-Flash reaction will produce 160-180  $\mu$ g of RNA from 1  $\mu$ g of the linearized 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 Specifications

**Storage:** Store only at  $-20^{\circ}\text{C}$  in a freezer without a defrost cycle. Do not store at  $-70^{\circ}\text{C}$ .

**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 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  $37^{\circ}\text{C}$ .

## 3. Kit contents

| Desc.   | Concentration          | Quantity           |
|---|------------------------|--------------------|
| The AmpliScribe™ T7-Flash™ Transcription Kit is available in 25- and 50-reaction sizes. |                        |                    |
| The 25-reaction size kit contains:  |                        |                    |
| AmpliScribe™ T7-Flash™ Enzyme Solution  |                        | 50 $\mu$ l         |
| 100 mM ATP, CTP, GTP, and UTP Solutions   |                        | each at 50 $\mu$ l |
| AmpliScribe™ T7-Flash™ 10X Reaction Buffer  |                        | 125 $\mu$ l        |
| 100 mM Dithiothreitol (DTT)   |                        | 50 $\mu$ l         |
| RiboGuard™ RNase Inhibitor  |                        | 15 $\mu$ l         |
| RNase-Free Water  |                        | 1 ml               |
| Control Template DNA  | @ 0.5 $\mu$ g/ $\mu$ l | 10 $\mu$ l         |
| RNase-Free DNase I  | @ 1 MBU/ $\mu$ l       | 25 $\mu$ l         |

## 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 T<sub>10</sub>E<sub>1</sub> (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).
- 2. Template Efficiency:** Linearized plasmid templates and PCR product templates which produce transcripts of equivalent sizes are utilized with equal efficiency by the AmpliScribe T7-Flash Transcription Kit. The Control DNA Template, 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 optimized 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 summarizes 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.

**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.

|                   |      | Incubation Time (minutes) |        |        |        |        |        |
|-------------------|------|---------------------------|--------|--------|--------|--------|--------|
|                   |      | 10                        | 15     | 20     | 30     | 60     | 120    |
| Template DNA (µg) | 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 |

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 AmpliScribe T7-Flash 10X 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. **Optimizing 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 DNA template, 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.

**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.

|   |       | Incubation Time (minutes) |                     |                     |                     |                      |
|---|-------|---------------------------|---------------------|---------------------|---------------------|----------------------|
|   |       | 30                        | 60                  | 120                 | 180                 | 240                  |
| Runoff<br>Template<br>DNA size<br>(bases) | 26 b  | 20 µg<br>2.33 nmol        | 36 µg<br>4.20 nmol  | 56 µg<br>6.53 nmol  | 88 µg<br>10.26 nmol | 104 µg<br>12.12 nmol |
|   | 47 b  | 24 µg<br>1.55 nmol        | 36 µg<br>2.32 nmol  | 62 µg<br>4.00 nmol  | 88 µg<br>5.67 nmol  | 104 µg<br>6.70 nmol  |
|   | 96 b  | 36 µg<br>1.14 nmol        | 60 µg<br>1.89 nmol  | 92 µg<br>2.90 nmol  | 128 µg<br>4.04 nmol | 144 µg<br>4.55 nmol  |
|   | 335 b | 76 µg<br>0.69 nmol        | 120 µg<br>1.09 nmol | 152 µg<br>1.38 nmol | ---                 | ---                  |

## 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   | RNase-Free water                                   |
| 1 µg   | linearized template DNA with appropriate promoter* |
| 2 µl   | AmpliScribe T7-Flash 10X 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   | AmpliScribe T7-Flash Enzyme Solution               |
| 20 µl  | Total reaction volume                              |

### 2. Incubate at 37°C for 30 minutes.

**Note:** Incubating the reaction at 42°C may increase yields by about 10%.

### 3. 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 minimizes 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 DNA template by a 6X scale-up of the standard 20- $\mu$ l reaction to 120  $\mu$ l using 6  $\mu$ g of control DNA template.

**Method 2** Scale-up all reaction components proportionally, *except the template DNA*.

This method minimizes 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 DNA template by an 8X scale-up of the standard 20- $\mu$ l reaction to 160  $\mu$ l using 1  $\mu$ g of control DNA template.

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  $\mu$ l (1 MBU) of RNase-Free DNase I to the standard 20- $\mu$ l 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 RNase-Free water, T<sub>10</sub>E<sub>11</sub>, 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 RNase-Free water, T<sub>10</sub>E<sub>11</sub>, or other suitable buffer.

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