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Simplifying Genomics

DuraScribe® T7 Transcription Kit

Cat. Nos. DS010910 and DS010925

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1. Introduction

The DuraScribe® T7 Transcription Kit* produces DuraScript® RNA that is resistant to RNase A degradation. This is accomplished by completely replacing the canonical CTP and UTP with 2′-Fluorine-dCTP (2′-F-dCTP) and 2′-Fluorine-dUTP (2′-F-dUTP) in the DuraScribe *in vitro* transcription reaction. The kit features DuraScribe T7 RNA Polymerase, a mutant T7 RNA Polymerase, that efficiently incorporates 2′-F-dCTP and 2′-F-dUTP, ATP and GTP into RNA. DuraScribe T7 RNA Polymerase utilizes the same transcription promoter sequences as standard T7 RNA Polymerase.

2. Product Designations and Kit Components

Product	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
DuraScribe T7 Transcription Kit	10 Rxns	DS010910	DuraScribe T7 Enzyme Mix (200 U/µL)	E0016-D1	20 µL
			DuraScribe T7 10X Reaction Buffer	SS000019-D1	20 µL
			ATP (50 mM)	SS000409-D1	20 µL
			GTP (50 mM)	SS000407-D1	20 µL
			2′-F-dCTP (50 mM)	SS000080-D1	20 µL
			2′-F-dUTP (50 mM)	SS000086-D1	20 µL
			DTT (100 mM)	SS000065-D2	25 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D3	25 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1.0 mL
			Control Template DNA (0.5 µg/µL)	SS000571-D1	10 µL
DuraScribe T7 Transcription Kit	25 Rxns	DS010925	DuraScribe T7 Enzyme Mix (200 U/µL)	E0016-D2	50 µL
			DuraScribe T7 10X Reaction Buffer	SS000019-D2	50 µL
			ATP (50 mM)	SS000409-D2	50 µL
			GTP (50 mM)	SS000407-D2	50 µL
			2′-F-dCTP (50 mM)	SS000080-D2	50 µL
			2′-F-dUTP (50 mM)	SS000086-D2	50 µL
			DTT (100 mM)	SS000065-D3	50 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D7	60 µL
			Nuclease-Free Water, Sterile	SS000772-D3	1.0 mL
			Control Template DNA (0.5 µg/µL)	SS000571-D2	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 DuraScribe Kit are free of detectable RNase activity, and all of the components except DNase I are free of detectable exo- and endonuclease activities.

4. Notes For Using The DuraScribe Kit

1. *Control Template DNA.* The Control Template DNA is a 4.2-kb linearized DNA plasmid that will produce a 1.4-kb DuraScript RNA transcript.
2. *Choice of Experimental DNA Template.* Linearized plasmids, double-stranded oligos and PCR products can all be used as template in a DuraScribe reaction. The template should be double-stranded with blunt or 5′-protruding ends. Templates with 3′-protruding ends can produce spurious transcripts. The template must contain a T7 transcription promoter.
3. *Template Efficiency.* Different templates may give different yields. When comparing yields of DuraScript RNA from different templates it is important to normalize the yields for the molar quantity of template included in the reaction and molar amount of DuraScript RNA produced (see Table 2). The control DuraScribe T7 reaction typically yields 40-60 µg (86-129 pmol) of a 1.4-kb DuraScript RNA per 1 µg (0.36 pmol) of the 4.2-kb Control Template DNA.
4. *Amount of Template.* The standard 20-µl, 4-hour DuraScribe reaction is optimized for using 1 µg of linearized DNA template. Lower amounts of DNA template can be used successfully in a DuraScribe reaction. However, the yield of DuraScript RNA will be reduced. Table 1 summarizes our experiences with reducing the amount of Control Template DNA in a standard DuraScribe reaction. Results may vary depending on the template used.

Table 1. Yield of DuraScript® RNA from reduced amounts of Control Template DNA in a standard 20 µl, 4-hour DuraScribe® T7 transcription reaction. Results may vary depending on the template.

Amount of Control Template DNA (µg)	Yield of DuraScript RNA (µg)
1 µg	40 – 60 µg
0.5 µg	30 – 40 µg
0.25 µg	20 – 30 µg
0.05 µg	7 – 10 µg
0.01 µg	2 – 4 µg

5. *DuraScribe Reaction Assembly.* Assemble a DuraScribe reaction at room temperature (22°C – 25°C). Assembly of the reaction at temperatures less than 22°C can result in formation of an insoluble precipitate in the reaction tube. Storing the DuraScribe T7 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.
6. *Reaction Time.* The standard DuraScribe reaction time is 4 hours. Increasing the reaction time to 6 hours may increase the yield of DuraScript RNA.
7. *Scale-up a DuraScribe Reaction.* A DuraScribe reaction can be scaled-up to produce even more DuraScript RNA. All reaction components, including the template DNA, should be scaled-up proportionally.

8. *Yield of DuraScript RNA.* The yield of DuraScript RNA is influenced by the amount of the DNA template in the reaction (see Note 4.4), the reaction time (see Note 4.6) and the size of the DuraScript RNA produced. When comparing the yield of DuraScript RNA produced from different templates, it is important to compare the moles of DuraScript RNA produced. As shown in Table 2, the number of micrograms of short DuraScript RNAs produced in a standard DuraScribe reaction is small compared to the microgram yield of long DuraScript transcripts. However, the number of moles of the short DuraScript RNAs produced is greater than the number of moles of the longer DuraScript RNAs because molar yields are inversely proportional to the transcript length.

Table 2. Yield of four DuraScript® RNA transcripts. In each experiment, 1 µg of a 3-kb DNA template, linearized at different sites, was transcribed in a standard DuraScribe T7 transcription reaction for 4 hours.

Size of the DuraScript RNA made	Yield of DuraScript RNA (µg)	Yield of DuraScript RNA (pmol)
2600 b	100 µg	116 pmol
1400 b	58 µg	124 pmol
330 b	18 µg	164 pmol
88 b	9 µg	307 pmol

9. *Nuclease Resistance of DuraScript RNA.* DuraScript RNA is resistant to RNase A and DNase I. DuraScript RNA will be digested by RNase T1. Other RNases and DNases have not been tested.
10. *Reverse Transcription of DuraScript RNA.* DuraScript RNA can be efficiently reverse transcribed into cDNA using MMLV, MMLV RNase H- and AMV Reverse Transcriptase enzymes.
11. *In vitro Translation of DuraScript RNA.* DuraScript RNA can not be translated in *in vitro* translation systems.
12. *Non-radioactive Labeling of DuraScript RNA.* See Appendix 1 for a recommended method for making fluorescently-labeled DuraScript RNA.

5. DuraScribe T7 Transcription Reaction

Warm the DuraScribe T7 Enzyme Mix to room temperature. Thaw the remaining DuraScribe T7 transcription reagents at room temperature. Thoroughly mix the thawed buffer and the nucleotide solutions.

Important! *If a precipitate is visible in the thawed DuraScribe T7 10X Reaction Buffer, heat the buffer to 37°C until the precipitate dissolves. Mix the buffer thoroughly. Keep the buffer at room temperature.*

Combine the following reaction components at room temperature in the order given:

x µL	Nuclease-Free Water, Sterile
1 µg	linearized template DNA with T7 promoter (see Note 4.4)
2 µL	DuraScribe T7 10X Reaction Buffer
2 µL	50 mM ATP
2 µL	50 mM GTP
2 µL	50 mM 2′-F-dCTP
2 µL	50 mM 2′-F-dUTP
2 µL	100 mM DTT
2 µL	DuraScribe T7 Enzyme Mix
20 µL	Total reaction volume

If desired, the reaction can be scaled up (see Note 4.7).

Incubate the reaction at 37°C for 4 hours (see Note 4.6).

After the reaction is complete, add 1 µL of RNase-Free DNase I to each 20 µL reaction. Incubate at 37°C for 15 minutes to remove the DNA template. The DuraScript RNA is resistant to DNase I digestion.

6. Purifying the DuraScript RNA

DuraScript RNA can be purified by either Method A or Method B described below.

Method A: For DuraScript 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.

Add one volume of 5 M ammonium acetate (21 µL for the standard DuraScribe reaction) to the completed reaction. Proceed to Method B, Step 2.

Method B: For DuraScript transcripts of all sizes, remove the unincorporated NTPs by spin column, then ethanol precipitate.

1. Following spin column, add 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol to the collected sample.
2. Incubate on ice or at –20°C for 15-30 minutes and collect by centrifugation at high speed (e.g., 10,000 x g) for 15 minutes at 4°C.
3. Remove the supernatant carefully with a pipette and gently rinse the pellet with 70% ethanol.
4. DuraScript 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.

7. 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.

8. Reference

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

9. Appendix

Fluorescent Labeling of DuraScript RNA

For optimal fluorescent labeling of DuraScript RNA we recommend incorporation of aminohexyl-ATP into the DuraScript RNA during the DuraScribe transcription reaction followed by post-transcriptional labeling with a Biotin-NHS ester or a fluorescent-NHS ester.

1. Warm the DuraScribe T7 Enzyme Mix to room temperature. Thaw the remaining DuraScribe T7 transcription reagents at room temperature. Thoroughly mix the thawed buffer and the nucleotide solutions.

   Important! *If a precipitate is visible in the thawed DuraScribe T7 10X Reaction Buffer, heat the buffer to 37°C until the precipitate dissolves. Mix the buffer thoroughly. Keep the buffer at room temperature.*

2. Combine the following reaction components at room temperature in the order given:

x µL	Nuclease-Free Water, Sterile
1 µg	linearized template DNA with T7 promoter (see Note 4.4)
2 µL	DuraScribe T7 10X Reaction Buffer
1.3 µL	50 mM ATP
3.4 µL	10 mM aminohexyl-ATP
2 µL	50 mM GTP
2 µL	50 mM 2′-F-dCTP
2 µL	50 mM 2′-F-dUTP
2 µL	100 mM DTT
2 µL	DuraScribe T7 Enzyme Mix
20 µL	Total reaction volume

3. Incubate at 37°C for 4 hours (see Note 4.6).
4. Purify the DuraScript RNA by ammonium acetate precipitation as described in Part 6.

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