Certificate of Analysis

pGEM® Express Positive Control Template:

Part No.	Size
P256A	10µg (2 × 5µg)

Description: The pGEM® Express Positive Control Template may be used to monitor the performance of the Riboprobe® Systems. The template was constructed by linearizing the pGEMEX®-1 Vector with ScaI.

DNA Concentration: 1.0mg/ml.

Storage Buffer: The pGEM® Express Positive Control Template is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Storage Conditions: See Product Information Label for storage recommendations. Avoid exposure to frequent temperature changes. See the expiration date on the product label.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

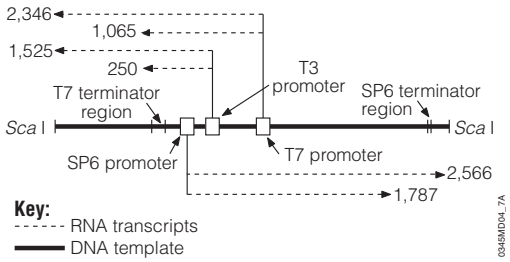

Figure 1. RNA transcripts (in bases) generated using the appropriate RNA polymerase and pGEM® Express Positive Control Template.

Quality Control Assays

Functional Assay: The pGEM® Express Positive Control Template is transcribed in vitro using SP6, T7 and T3 polymerases, and the resulting transcripts are analyzed by agarose gel electrophoresis. The transcript sizes (given in bases) are as follows:

RNA Polymerase	Transcript Sizes (in bases)
SP6	2566, 1787
T7	2346, 1065
T3	1525, 250

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIP256

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Usage Information

1. Description

Transcription with SP6 RNA Polymerase produces RNA transcripts of 1,787 bases and 2,566 bases. The shorter transcript (1,787 bases) is due to the presence of an SP6 terminator region within the template. The terminator is approximately 50% efficient. Transcription of the template with T7 RNA Polymerase results in transcripts of 1,065 bases and 2,346 bases. The shorter transcript (1,065 bases) is due to the presence of a T7 terminator region within the template. The terminator is approximately 70–80% efficient. Transcription with T3 RNA Polymerase results in transcripts of 250 bases and 1,525 bases. The shorter transcript (250 bases) is again due to the presence of the T7 terminator, which is recognized to some extent by T3 RNA Polymerase (Figure 1).

2. pGEM® Express Positive Control Protocol

Reagents to Be Supplied by the User

(Solution compositions are provided in Section III. Products denoted with an asterisk (*) are available from Promega.)

- Transcription Optimized 5X Buffer* (Cat.# P1181)
- DTT, 100mM* (Cat.# P1171)
- RNasin® Ribonuclease Inhibitor* (Cat.# N2111)
- rNTPs, 2.5mM each* (Cat.# P1221)
- SP6, T7 or T3 RNA Polymerase* (Cat.# P1085, P2075 or P2083)
- Nuclease-Free Water* (Cat.# P1193)
- RNA sample buffer
- TAE running buffer
- RNA loading buffer
- **Optional:** RQ1 RNase-Free DNase* (Cat.# M6101)

A. Transcription Reaction

We recommend setting up an unlabeled positive control reaction to monitor transcription reactions performed using the Riboprobe® Systems.

1. Assemble the following reaction components at room temperature in the order shown:

Transcription Optimized 5X Buffer	4µl
DTT, 100mM	2µl
RNasin® Ribonuclease Inhibitor	20u
ATP, CTP, GTP and UTP (2.5mM each) (prepared by mixing equal volumes of 10mM rNTP stocks)	4µl
pGEM® Express Positive Control Template	1µg
SP6, T7 or T3 RNA Polymerase (at 15–20u/µl)	1µl
Nuclease-Free Water to final volume of	20µl

2. Incubate for 60 minutes at 37–40°C.
3. **Optional:** To remove the DNA template, add RQ1 RNase-Free DNase to a concentration of 1u/µg control DNA template. Incubate for 15 minutes at 37°C.

   Note: If the template is not removed, a high molecular weight band (approximately 4kb) will be present when visualizing the reaction products on a gel.

B. Gel Analysis

The reaction may be run on a 1% nondenaturing agarose gel using TAE 1X running buffer. To a 5µl aliquot of the transcription reaction, add 15µl of RNA sample buffer. Add 2–5µl of RNA loading buffer and heat the sample for 5–10 minutes at 65–70°C prior to loading. Run the gel under standard conditions used for analysis of DNA samples. A standard "minigel" may be run for 20 minutes at 60V. RNA Markers (Cat.# G3191), which are single-stranded, may be used to determine the sizes of the products. SP6 RNA Polymerase produces two transcripts of 1,787 bases and 2,566 bases. Two bands should be visible if T7 RNA Polymerase is used: 1,065 bases and 2,346 bases. T3 RNA Polymerase produces bands of 250 bases and 1,525 bases.

For a more thorough discussion of transcription, please request the *Riboprobe® in vitro Transcription Systems Technical Manual* #TM016.

3. Composition of Buffers and Solutions

RNA loading buffer

50%	glycerol
1mM	EDTA
0.4%	bromophenol blue
1mg/ml	ethidium bromide

Make up 20ml using a very-high-grade, RNase-free glycerol. Dispense into single-use aliquots and store at –20°C.

RNA sample buffer

10.0ml	deionized formamide
3.5ml	37% formaldehyde
2.0ml	MOPS buffer

Dispense into single-use aliquots and store at –20°C in tightly sealed screw-cap tubes. The buffer can be stored for up to 6 months. Do not freeze-thaw the buffer more than twice.

5X MOPS buffer

0.2M	MOPS (pH 7.0)
50mM	sodium acetate
5mM	EDTA (pH 8.0)

TAE running buffer (50X stock)

242g	Tris-base
57.1ml	glacial acetic acid
100ml	0.5M EDTA (pH 8.0)

Adjust to pH 7.9 and bring to a final volume of 1 liter with water.

Transcription Optimized 5X Buffer

200mM	Tris-HCl (pH 7.5)
30mM	$MgCl_2$
10mM	spermidine
50mM	NaCl

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