Promega

1. Description

The T7 RiboMAX™ Express Large Scale RNA Production System is an in vitro transcription system designed for the consistent production of milligram amounts of RNA in a short amount of time. This is about 10- to 20-fold more RNA than produced in a standard Riboprobe® System transcription reaction. The T7 RiboMAX™ Express System reaction differs from those of the Riboprobe® System in three primary ways: a HEPES (pH 7.5) (1) buffer is used rather than a Tris-HCl (pH 7.9) buffer, NTP and magnesium concentrations are elevated and inorganic pyrophosphatase is included in the reaction (2).

The T7 RiboMAX™ Express Large Scale RNA Production System differs from the standard RiboMAX™ System in two primary ways:

Time savings: The T7 RiboMAX™ Express System produces milligram amounts of RNA in as little as 30 minutes rather than the 2–4 hours required with other commercially available systems including the original RiboMAX™ System.

Convenience: The rNTPs and Transcription Buffer are combined, minimizing pipetting errors and setup time.

The production of large amounts of RNA is potentially valuable for in vitro translation; synthesis of tRNA, rRNA and other small, functional RNAs; transcription of RNA virus genomes and ribozymes; and for production of substrates for studying RNA splicing, RNA secondary structure, antisense RNA and RNA:protein interactions. Because the T7 RiboMAX™ Express System produces large quantities of RNA, this system is not recommended for generating high-specific-activity RNA probes. The amount of radiolabeled nucleotide required to produce this type of probe would be prohibitively expensive. The T7 RiboMAX™ Express System is not recommended for the production of capped RNA.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
T7 RiboMAX™ Express Large Scale RNA Production System	**1 system**	**P1320**

Each system contains sufficient reagents for 50 standard 20µl reactions. Includes:

- 100µl Enzyme Mix T7 Express
- 500µl RiboMAX™ Express T7 2X Buffer
- 110 units RQ1 RNase-Free DNase, 1U/µl
- 2 × 5µg pGEM® Express Positive Control Template, 1mg/ml
- 1ml 3M Sodium Acetate (pH 5.2)
- 1.25ml Nuclease-Free Water

Storage Conditions: Store all components at –20°C.

Table 1. Commonly Used Restriction Enzymes That Generate 3´ Overhangs.

AatII	ApaI	BanII
BglI	Bsp1286I	BstXI
CfoI	HaeII	HgiAI
HhaI	KpnI	PstI
PvuI	SacI	SacII
SfiI	SphI	

PCR-generated DNA containing an appropriate phage promoter can be used in transcription reactions. The phage promoter sequences can be incorporated into the DNA by using primers that flank the phage promoter sequences in the vector or by having the promoter sequence within the 5´ oligomer used in the PCR. The resulting PCR-generated DNA can be purified using the Wizard® PCR Preps DNA Purification System (Cat.# A7170) or Wizard® SV Gel and PCR Clean-Up System (Cat.# A9282).

The purified linear DNA should be examined by agarose or polyacrylamide gel electrophoresis prior to transcription to verify complete linearization and to ensure the presence of a clean (nondegraded) DNA fragment of the expected size.

4. Transcription Protocol

This protocol was developed by combining and modifying two published protocols that use HEPES buffer (1) and yeast inorganic pyrophosphatase (2). The pGEM® Express Positive Control Template DNA supplied with the system produces transcripts that are 1.1kb and 2.3kb in length. **The transcripts produced from the pGEM® Express Positive Control Template are not suitable for in vitro translation.**

4.A. Synthesizing Large Quantities of RNA

1. Set up the appropriate reaction size at room temperature. Add the reaction components in the order shown; be careful to dissolve the DNA template in water before adding it to the reaction.

T7 Reaction Components	Sample Reaction	Control Reaction
RiboMAX™ Express T7 2X Buffer*	10µl	10µl
linear DNA template (1µg total)	1–8µl	—
pGEM® Express Positive Control Template (1µg)	—	1µl
Nuclease-Free Water	0–7µl	7µl
Enzyme Mix, T7 Express	2µl	2µl
final volume	**20µl**	**20µl**

*Frozen RiboMAX Express T7 2X Buffer will contain a precipitate that can be dissolved by warming the buffer at 37°C and mixing well.

2. Mix gently and incubate at 37°C for 30 minutes.

Note: Do NOT freeze transcription reactions. After the transcription reaction is complete, proceed directly to the DNase step or removal of unincoporated rNTPs.