Making RNA in vitro

Overview

When you want to make RNA in vitro to use as a probe in a hybridization or to produce RNA for the study of RNA structure/function, Promega has the system you need. The Riboprobe® in vitro Transcription System will produce microgram quantities of either labeled or unlabeled RNA for use as probes or templates for in vitro translations. The RiboMAX™ Large Scale RNA Production System is a scalable reaction system allowing the production of up to milligram quantities of transcripts. The T7 RiboMAX™ Express System uses an optimized master mix to produce the highest quantities of uncapped RNAs from a T7 promoter. The standard RiboMAX™ Systems can be used to produce capped RNAs with the addition of Ribo m^7G Cap Analog. Which system to use is determined by the quantity of RNA required. All the systems produce RNA in vitro by transcribing RNA from DNA plasmid templates using either the SP6 or T7 RNA Polymerases. These RNA polymerases recognize a specific DNA sequence and begin transcription from a defined location. Many standard cloning vectors, like the pGEM® Vectors, have the SP6 or T7 RNA polymerase recognition sites conveniently located outside the multiple cloning sites. These vectors have dual opposed SP6 or T7 promoters, allowing the transcription of either strand of the cDNA.

For a complete listing of pGEM® Vectors, go to: **www.promega.com/vectors/cloning_vectors.htm** pGEM® Vectors have dual, opposed SP6 and T7 promoters ideal for use with phage RNA polymerase-based in vitro transcription. Choose the vector that best suits your subcloning needs. If you are using amplification to generate your insert, try either the pGEM®-T or pGEM®-T Easy Vector Systems (Cat.# A3600 and A1380).

Need **micrograms** of RNA? **Riboprobe®** in vitro Transcription Systems

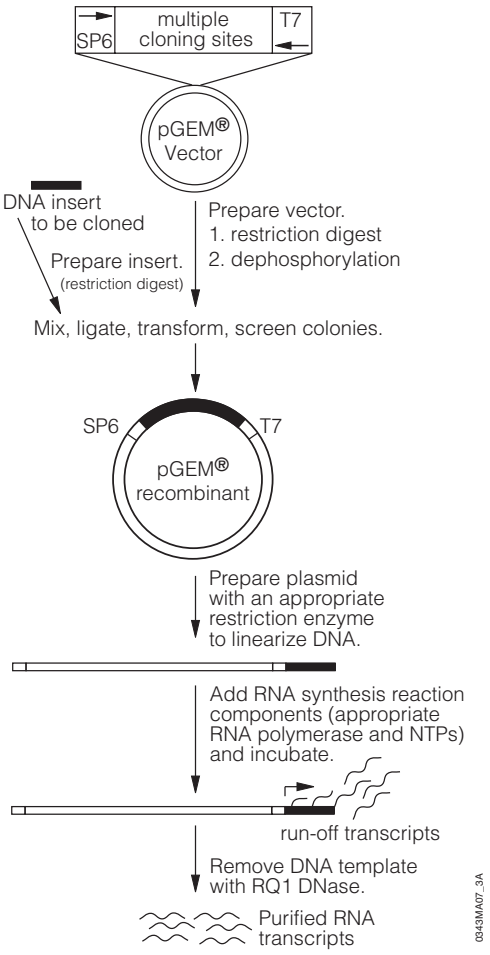
Need **labeled probes?** **Riboprobe®** in vitro Transcription Systems

Need **milligrams** of RNA? **RiboMAX™** Large Scale RNA Production Systems

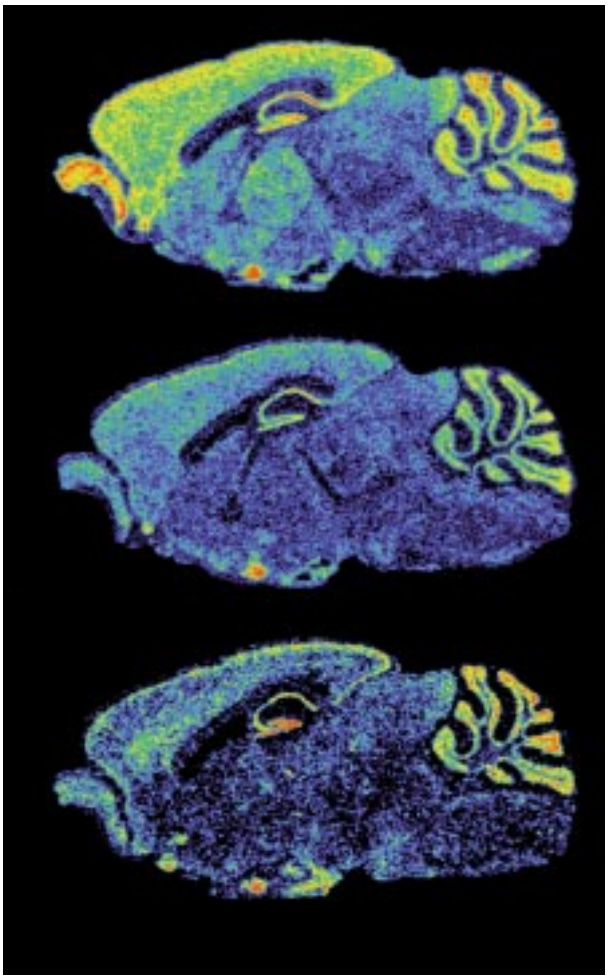
Application	Riboprobe® Systems	RiboMAX™ Systems
Membrane Hybridization	+++	+
RNase Protection Assay	+++	+
in situ Hybridization	+++	+
Library Screening	+++	+
Transcription Mapping	+++	+
in vitro Translation	+++	+++
Oocyte Injection	+	+++
Mammalian Transfection	+	+++
Ribozyme Generation	+	+++
Synthesis of Small RNA Molecules	+	+++
RNAi	+	+++

Riboprobe® in vitro Transcription Systems

The original system for making RNA probes was the Riboprobe® in vitro Transcription System. The Riboprobe Systems can be used to make microgram quantities of RNA for use as probes for membrane-based and in situ hybridizations. The system is routinely used for incorporation of radioactive nucleotides (1) and can be used to make biotinylated (2) and digoxygenin-tagged (3) probes. Non-radioactive transcripts have been used to make standards for quantitative RT-PCR (4).



Schematic of the Riboprobe® in vitro Transcription System.



Pseudocolor images of mPergene expression in mouse brain. Adjacent, parasagittal sections through the SCN were processed for in situ hybridization with cRNA probes to detect *mPer1* (top), *mPer2* (center) and *mPer3* (bottom) mRNAs. Rostral is to the left, and ventral is down in each image. Note the high intensity of hybridization signal (red in this pseudocolor image) in the SCN of the ventral hypothalamus. (Images derived from Shearman, L.P., Reppert, S.M. and Weaver, D.R. (1999) Techniques for analyzing gene expression in the mammalian circadian clock. *Neural Notes* **14**,17–19).

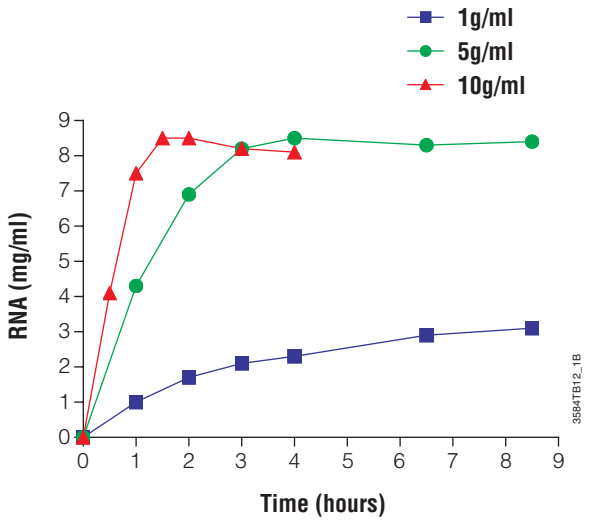
Riboprobe® in vitro Transcription System
Protocol available at:
www.promega.com/tbs/tm016/tm016.html
T7 Cat.# P1440
SP6 Cat.# P1420
T3 Cat.# P1430
SP6/T7 Cat.# P1460
T7/T3 Cat.# P1450

RiboMAX™ Large Scale RNA Production Systems

The RiboMAX™ Large Scale RNA Production Systems allow you to produce milligram quantities of RNA transcripts from standard DNA templates. Such large quantities are possible due to the RiboMAX™ Enzyme Mix. The enzyme mix contains RNA polymerase, yeast inorganic pyrophosphatase and Recombinant RNasin® Ribonuclease Inhibitor. As the RNA polymerase incorporates nucleotides, pyrophosphate accumulates in the reaction. Excess pyrophosphate will eventually inhibit the RNA polymerase by slowing or stoping incorporation. Addition of the yeast inorganic pyrophosphate converts the pyrophosphate to phosphate allowing the reaction to continue, resulting in milligram quantities of template. Use of the original RiboMAX™ Systems allow you to substitute labeled nucleotides such as biotinylated (5) or fluorescently labeled nucleotides (6) for unlabeled nucleotides. The original systems are also ideal for making capped transcripts with the use of the Ribo m^7G Cap Analog (Cat.# P1711) for maximum efficiency in an uncoupled in vitro translation reaction. If your objective is to make the maximum amount of unlabeled, uncapped RNA, the T7 RiboMAX™ Express System is the system to use.

RiboMAX™ Large Scale RNA Production Systems
SP6 Cat.# P1280
T7 Cat.# P1300
Protocol available at:
www.promega.com/tbs/tb166/tb166.html
Citations available at:
www.promega.com/citations/

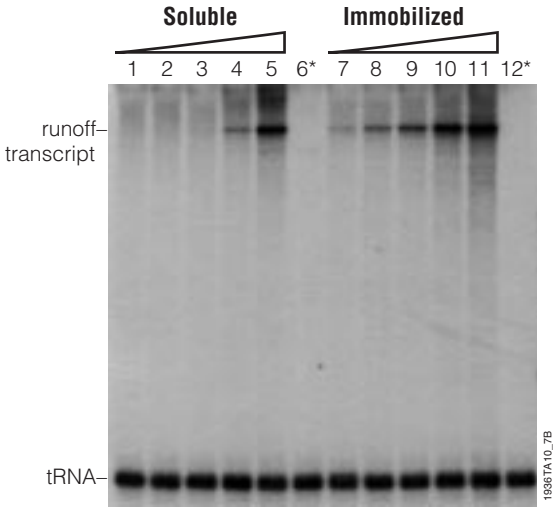
T7 RiboMAX™ Express Large Scale RNA Production System
Cat.# P1320
Protocol available at:
www.promega.com/tbs/tb298/tb298.html
Featured in Promega Notes 80:
www.promega.com/pnotes/



Template DNA concentrations versus time. Transcription reactions were prepared as described in Technical Bulletin #TB298, containing 1, 5 or 10µg/ml of pGEM® Express Positive Control Template (final concentration) per 20µl reaction volume and adding the Enzyme Mix last. The reactions were immediately dispensed and placed at 37°C. Duplicate reactions were removed at the times indicated, placed on ice and quenched with 180µl of TE buffer. A portion of each reaction was diluted 1,000-fold and assayed for RNA content with the RiboGreen® Kit (Molecular Probes). For more information, see Adams, J., Patterson, B. and Wautlet, M. (2002) Fast & efficient production of RNA with the T7 RiboMAX™ Express System. *Promega Notes* **80**, 5–9.

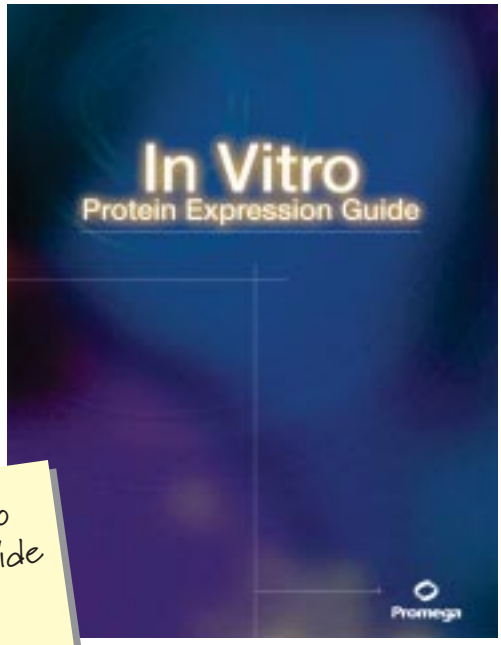
Eukaryotic in vitro Transcription

HeLa cell nuclear extracts are commonly used to study promoter activation and eukaryotic transcription factor function. Promega produces one of the most consistent sources of HeLa Nuclear Extracts to insure continuity from one experiment to the next. You can purchase the extract alone or with all components needed for eukaryotic in vitro transcription.



Transcription with continuous labeling of an immobilized template.
A biotinylated template was immobilized on Streptavidin MagneSphere® Paramagnetic Particles and in vitro transcribed with the HeLaScribe® Nuclear Extract. Full details available in Liu, M. and Price, D.H. (1997) In vitro transcription on DNA templates immobilized to Streptavidin MagneSphere® Paramagnetic Particles. *Promega Notes* **64**, 21–26.

HeLaScribe® Nuclear Extract in vitro Transcription System
Cat.# E3110
Protocol available at:
www.promega.com/tbs/tb123/tb123.html
HeLaScribe® Nuclear Extract
Cat.# E3091
Citations available at:
www.promega.com/citations/



Free in vitro Expression Guide (BR053) available!

Making RNA in vitro

Riboprobe® Systems	Size	Cat.#
Riboprobe® System–SP6	1 system	P1420
Riboprobe® System–T3	1 system	P1430
Riboprobe® System–T7	1 system	P1440
Riboprobe® Combination System T3/T7 RNA Polymerase	1 system	P1450
Riboprobe® Combination System SP6/T7 RNA Polymerase	1 system	P1460

For Laboratory Use.

Each system has sufficient reagents for 25 × 20µl reactions. Combination systems contain 25 reactions total, not 25 reactions with each RNA polymerase.

RiboMAX™ Large Scale RNA Production Systems	Size	Cat.#
RiboMAX™ Large Scale RNA Production System—SP6	1 system	P1280
RiboMAX™ Large Scale RNA Production System—T7	1 system	P1300

For Laboratory Use.

Each system has sufficient reagents for a single 1ml transcription reaction, 10 × 100µl reactions, or 50 × 20µl reactions. Designed for use with Ribo m^7G Cap Analog (Cat.# P1711) and can be used with modified nucleotides.

T7 RiboMAX™ Express Large Scale RNA Production System	Size	Cat.#
T7 RiboMAX™ Express Large Scale RNA Production System	1 system	P1320

For Laboratory Use.

Each system has sufficient reagents for a single 1ml transcription reaction, 10 × 100µl reactions, or 50 × 20µl reactions.

Phage RNA Polymerases*	Size	Cat.#
SP6 RNA Polymerase (10–20u/µl)	1,000 units	P1085
	5,000 units	P1081
SP6 RNA Polymerase (80u/µl)	2,500 units	P4084
T3 RNA Polymerase (10–20u/µl)	1,000 units	P2083
T3 RNA Polymerase (80u/µl)	2,500 units	P4024
T7 RNA Polymerase (10–20u/µl)	1,000 units	P2075
	5,000 units	P2077
T7 RNA Polymerase (80u/µl)	10,000 units	P4074

For Laboratory Use.

* One unit is defined as the amount of enzyme required to catalyze the incorporation of 5nmol of CTP into acid-insoluble product in 60 minutes at 37°C in a total volume of 100µl under optimal reaction conditions with 2µg supercoiled vector DNA. Most other RNA polymerases sold commercially use incorporation into 1nmol of acid-insoluble product. Therefore, 1 Promega unit may equal 5 competitor units.

Riboprobe® and RiboMAX™ Accessory Products	Size	Cat.#
Ribo m^7G Cap Analog	10 A_{254} units	P1711
	25 A_{254} units	P1712
Set of rNTPs*, 10mM each	0.5ml each	P1221
Set of rNTPs*, 100mM each	4 × 400µl each	E6000
Riboprobe® System Buffers*	1 kit	P1121
pGEM® Express Positive Control Template	10µg (2 × 5µg)	P2561

* For Laboratory Use.

Eukaryotic in vitro Transcription	Size	Cat.#
HeLaScribe® Nuclear Extract in vitro Transcription System	40 reactions	E3110
HeLaScribe® Nuclear Extract, in vitro Transcription Grade	40 reactions	E3091
	160 reactions	E3092
HeLaScribe® Nuclear Extract Positive Control DNA	300ng	E3621

RNase Protection	Size	Cat.#
Recombinant RNasin® Ribonuclease Inhibitor	2,500 units	N2511
	10,000 units	N2515
RNasin® Plus RNase Inhibitor	2,500 units	N2611
	10,000 units	N2615

References

1. *Riboprobe® in vitro Transcription System Technical Manual,* #TM016, Promega Corporation.
2. Grousset, C. *et al.* (2000) A mechanism for translationally coupled mRNA turnover: Interaction between the poly(A) tail and a c-fos RNA coding determinant via a protein complex. *Cell* **103**, 29–40.
3. Washburn, C.P. *et al.* (2002) Serotonergic Raphe neurons express TASK channel transcripts and TASK-like pH- and halothane-sensitive K+ conductance. *J. Neurosci.* **22**, 1256–1265.
4. Hodzic, E. *et al.* (2002) *Borrelia burgdorferi* population kinetics and selected gene expression at the host-vector interface. *Infect. Immun.* **70**, 3382–3388.
5. Su, C. and Sordillo, L.M. (1998) A simple method to enrich mRNA from total prokaryotic RNA. *Mol. Biotechnol.* **10**, 83–85.
6. Gawn, J.M. and Greaves, R.F. (2002) Absence of IE1 p72 protein function during low-multiplicity infection by human cytomegalovirus results in a broad block to viral delayed-early gene expression. *J. Virol.* **76**, 4441–4455.