

RiboMAX™ Large Scale RNA Production Systems – SP6 and T7



Technical Bulletin No. 166

INSTRUCTIONS FOR USE OF PRODUCTS P1280 AND P1300. PLEASE DISCARD PREVIOUS VERSIONS.

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I. Description

In vitro transcription reactions are widely used to synthesize microgram amounts of RNA probes from recombinant DNA templates. Most transcription reactions designed to generate RNA probes are optimized to maximize incorporation of radio-labeled ribonucleotides rather than produce large amounts of RNA. However, in vitro transcription also is used for many other applications that require larger amounts of biologically active RNA. The production of large amounts of RNA is potentially valuable for in vitro translation; for synthesis of tRNA, rRNA, other small, functional RNAs, RNA virus genomes and ribozymes; and for production of substrates for studies of RNA splicing, RNA secondary structure, antisense RNA and RNA-protein interactions.

The RiboMAX™ Large Scale RNA Production Systems^(a,b,c,d) produce milligram amounts of RNA. Transcripts up to 14kb have been generated using the RiboMAX™ Systems; however, they are more commonly used to generate transcripts up to 5–6kb in size. The RiboMAX™ Systems consistently produce 2–5mg/ml of RNA in a 1ml reaction, about 10- to 20-fold more RNA than is produced with the standard Riboprobe® System^(b,d) transcription reaction. The RiboMAX™ System reactions

differ from those of the Riboprobe® Systems in three primary ways: a HEPES (pH 7.5) buffer is used rather than a Tris-HCl (pH 7.9) buffer; rNTP and magnesium concentrations are elevated at levels appropriate for either SP6 or T7 RNA Polymerase (1); and yeast inorganic pyrophosphatase is included in the reaction (2). Both systems include Recombinant RNasin® Ribonuclease Inhibitor^(b,d).

An additional advantage of the RiboMAX™ Systems is that the RNAs synthesized are of higher quality for in vitro translation in rabbit reticulocyte translation systems than RNA synthesized by standard methods (3). This enhanced “translatability” is especially evident at high RNA concentrations that normally inhibit in vitro translation (4,5). While the reasons for the observed improvements in RNA production and translation efficiency are not entirely clear, the RiboMAX™ Systems are useful to researchers wishing to produce large amounts of RNA for in vitro translation. The reduction of components inhibitory to translation also may be advantageous for other applications requiring biologically active RNA.

Because the RiboMAX™ Systems produce large quantities of RNA, these systems are not recommended for the generation of high specific activity RNA probes. The amount of radiolabeled nucleotide required to produce this type of probe would be prohibitively expensive.

II. Product Components

Product	Cat.#
RiboMAX™ Large Scale RNA Production System – SP6	P1280
RiboMAX™ Large Scale RNA Production System – T7	P1300

For Laboratory Use. Each system contains sufficient reagents for a 1 ml reaction or 50 standard 20µl reactions. Includes:

- 120µl Enzyme Mix (RNA Polymerase, Recombinant RNasin® Ribonuclease Inhibitor and Yeast Inorganic Pyrophosphatase)
- 240µl Transcription 5X Buffer
- 100µl Each of 4 rNTPs, 100mM
- 110u RQ1 RNase-Free DNase, 1u/µl
- 10µl Linear Control DNA, 1mg/ml
- 1ml 3M Sodium Acetate (pH 5.2)
- 1.25ml Nuclease-Free Water
- 1 Protocol

Storage and Stability: Store all components at –20°C. The RiboMAX™ Systems are stable for 6 months if stored and handled properly. Avoid multiple freeze-thaw cycles.

III. DNA Template Preparation

Materials to Be Supplied by the User

(Solution compositions are provided in Section VI.)

- chloroform:isoamyl alcohol (24:1)
- TE-saturated (pH 8.0) phenol:chloroform:isoamyl alcohol (25:24:1)
- ethanol (70% and 95%)

A. Template Linearization

Optimal RNA yields depend on starting with a high-quality DNA template. Both cesium chloride purification and the Wizard® *Plus* Minipreps DNA Purification System^(e) (Cat.# A7100) yield DNA suitable for transcription reactions. It is important that no RNase be present in the DNA. If the presence of RNase is suspected, treat the DNA with Proteinase K (100µg/ml) and SDS (0.5%) in 50mM Tris-HCl (pH 7.5), 5mM CaCl₂ for 30 minutes at 37°C (6). Purify the DNA further by extraction with TE-saturated (pH 8.0) phenol:chloroform:isoamyl alcohol (25:24:1) and ethanol precipitation (Section IV.B, Steps 3–6).

DNA templates are usually linearized prior to *in vitro* transcription to produce RNA of defined length. Linearize the DNA by digestion with an appropriate restriction endonuclease followed by an appropriate clean-up procedure, such as phenol extraction followed by ethanol precipitation, or the Wizard® DNA Clean-Up System^(e) (Cat.# A7280). It is useful to start with at least 30% more DNA than is required for the transcription reaction to allow for DNA loss during purification and for visualization by gel electrophoresis.

It is important to avoid the use of restriction enzymes that produce 3' overhangs (see Table 1). Extraneous transcripts have been reported to appear, in addition to the expected transcript, when such templates are transcribed (7). The extraneous transcripts can contain sequences complementary to the expected transcript as well as sequences corresponding to the vector DNA. If these enzymes must be used, the linearized template ends can be made blunt using DNA Polymerase I Large (Klenow) Fragment (Cat.# M2201) prior to transcription (7; Section III.B).

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


<i>Aat</i> II	<i>Apa</i> I	<i>Ban</i> II
<i>Bgl</i> I	<i>Bsp</i> 1286 I	<i>Bst</i> X I
<i>Cfo</i> I	<i>Hae</i> II	<i>Hgi</i> A I
<i>Hha</i> I	<i>Kpn</i> I	<i>Pst</i> I
<i>Pvu</i> I	<i>Sac</i> I	<i>Sac</i> II
<i>Sfi</i> I	<i>Sph</i> I	

PCR^(f)-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 reaction. The resulting PCR-generated DNA can be purified using the Wizard® PCR Preps DNA Purification System^(g) (Cat.# A7170).

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.

B. Conversion of a 3' Overhang to a Blunt End

1. Set up a standard *in vitro* transcription reaction (Section IV.A) minus the nucleotides and RNA polymerase.
2. Add DNA Polymerase I Large (Klenow) Fragment at a concentration of 5u/µg and incubate the reaction mixture for 15 minutes at 22°C.
3. Proceed with the transcription reaction by adding the nucleotide mix and RNA polymerase.

 **Avoid the use**
of restriction enzymes that
produce 3' overhangs.

Note: If there are no alternative restriction sites, convert the 3' overhang to a blunt end using the 3'→5' exonuclease activity of DNA Polymerase I Large (Klenow) Fragment.

IV. Transcription Protocol

This protocol was developed by combining and modifying two published protocols that use HEPES buffer (1) and yeast inorganic pyrophosphatase (2). The development of this system, a comparison to a standard transcription protocol (8), and data demonstrating the enhanced “translatability” of RNA generated by this system are described in reference 3.

The Linear Control DNA supplied with each system contains a luciferase gene under the control of the appropriate SP6 or T7 RNA Polymerase promoter. This DNA produces a transcript approximately 1,800 bases in length. Since luciferase must be full-length to show activity, transcription and translation of the Control DNA followed by a luciferase assay is a convenient means to verify that full-length transcripts have been generated.

A. Synthesis of up to Milligram Quantities of RNA

1. Set up the appropriate reaction for SP6 or T7 RNA Polymerase at room temperature. Add the reaction components in the order shown, being careful to dissolve the DNA template in water before addition to the reaction.

For convenience, mix equal volumes of the 4 individual 100mM rNTPs provided to produce a 100mM solution that is 25mM for each nucleotide.

SP6 Reaction Components	Sample Reaction	Control Reaction
SP6 Transcription 5X Buffer	20µl	4µl
rNTPs (25mM ATP, CTP, GTP, UTP)	20µl	4µl
linear DNA template (5–10µg total)		(Control DNA) 1µl
plus Nuclease-Free Water	50µl	(Water) 9µl
Enzyme Mix (SP6)	10µl	2µl
final volume	100µl	20.0µl

T7 Reaction Components	Sample Reaction	Control Reaction
T7 Transcription 5X Buffer	20µl	4µl
rNTPs (25mM ATP, CTP, GTP, UTP)	30µl	6µl
linear DNA template (5–10µg total)		(Control DNA) 1µl
plus Nuclease-Free Water	40µl	(Water) 7µl
Enzyme Mix (T7)	10µl	2µl
final volume	100µl	20µl

Larger-scale reactions may be performed by increasing the volumes proportionally.

2. Gently pipet the reaction to mix and incubate at 37°C for 2–4 hours.

B. Removal of the DNA Template Following Transcription

The DNA template may be removed by digestion with DNase following the transcription reaction. Promega’s RQ1 RNase-Free DNase (Cat.# M6101) has been tested for its ability to degrade DNA while maintaining the integrity of RNA. For some uses, it may not be necessary to remove the DNA template. For example, 1–10µl of a 1:10 to 1:50 dilution of the transcription reaction can be added directly to a rabbit reticulocyte in vitro translation reaction. The RNA should be DNase treated if accurate RNA concentration determination is desired or to remove potentially inhibitory or interfering components.

Note: DNA can precipitate in the presence of spermidine at colder temperatures.

Note: These reactions can be scaled up or down to suit your template production requirements. For example, a 1ml reaction will typically produce 2–5mg of RNA in 2–4 hours.

Materials to Be Supplied by the User

(Solution compositions are provided in Section VI.)

- TE-saturated (pH 4.5) phenol:chloroform:isoamyl alcohol (25:24:1)
- chloroform:isoamyl alcohol (24:1)
- isopropanol
- ethanol (70% and 95%)

After performing the in vitro transcription reaction:

1. Add RQ1 RNase-Free DNase to a concentration of 1u/μg of template DNA.
2. Incubate for 15 minutes at 37°C.
3. Extract with 1 volume of TE-saturated (pH 4.5) phenol:chloroform:isoamyl alcohol (25:24:1). Vortex for 1 minute and spin at top speed in a microcentrifuge for 2 minutes.
4. Transfer the upper, aqueous phase to a fresh tube and add 1 volume of chloroform:isoamyl alcohol (24:1). Vortex for 1 minute and centrifuge as described in Step 3. At this point, unincorporated nucleotides may be removed (Section IV.C) or the RNA may be precipitated directly (Step 5, below).
5. Transfer the upper, aqueous phase to a fresh tube. Any transferred chloroform can be removed by performing a quick spin (10 seconds) in a microcentrifuge followed by removal of the bottom phase with a micropipet. Add 0.1 volume of 3M Sodium Acetate (pH 5.2), and 1 volume of isopropanol or 2.5 volumes of 95% ethanol. Mix and place on ice for 2–5 minutes. Spin at top speed in a microcentrifuge for 10 minutes.
6. Carefully pour off or aspirate the supernatant and wash the pellet with 1ml of 70% ethanol. Dry the pellet under vacuum and suspend the RNA sample in TE buffer or Nuclease-Free Water to a volume identical to that of the transcription reaction. Store at –70°C.

C. Chromatographic Removal of Unincorporated Nucleotides

Unincorporated nucleotides can be removed from the RNA transcript by several isopropanol or ethanol precipitation steps or, most effectively, by size exclusion chromatography through a small Sephadex® G-50 or G-100 column in 10mM Tris-HCl (pH 7.5) and 0.1% SDS. Once the RNA has been separated from the unincorporated nucleotides, the sample can be ethanol precipitated as described in Section IV.B, Steps 5 and 6. Store at –70°C.

D. Determination of RNA Concentration and Visualization by Electrophoresis**Materials to Be Supplied by the User**

(Solution compositions are provided in Section VI.)

- RNA loading buffer
- RNA sample buffer

After removal of the DNA template and unincorporated nucleotides, the RNA concentration can be quantitated most easily by ultraviolet light absorbance. A 1:100 to 1:300 dilution of the RNA is prepared and the absorbance is read at a wavelength of 260nm. One A_{260} unit equals approximately 40μg/ml of RNA. Alternatively, trace amounts of radiolabeled nucleotide can be added to the reac-

Note: If DNase treatment is not performed, proceed to Step 3.

Note: Isopropanol precipitates less of the unincorporated nucleotides than ethanol.

tion ($[^{32}\text{P}]\text{rUTP}$, for example), and the percent incorporation can be determined by TCA precipitation. However, this is unnecessary for most applications other than probe synthesis.

The DNase-treated *in vitro* transcript can be examined by denaturing gel electrophoresis to determine the accuracy of the A_{260} quantitation and the integrity of the full-length transcript. Including RNA Markers^(d) (Cat.# G3191) allows for the determination of the RNA transcript size. The Linear Control DNA produces a transcript approximately 1,800 bases in length. This RNA can be added to a translation extract (rabbit reticulocyte or wheat germ) and the expression of functional luciferase can be determined in a non-radioactive assay using Promega's Luciferase Assay System^(h,i) (Cat.# E1500).

Prepare either an agarose gel in 1X TAE containing 0.5 $\mu\text{g}/\text{ml}$ ethidium bromide or an acrylamide minigel, depending upon the length of the transcript involved (0.7–2.0% agarose for transcripts from 200 to several thousand nucleotides; 5% acrylamide for transcripts from 50–1,000 nucleotides). While denaturing gels (containing formaldehyde, glyoxal or 8M urea) provide the greatest resolution of the denatured RNA, we have found that perfectly acceptable results usually can be obtained using nondenaturing gels loaded with RNA denatured in a formaldehyde/formamide sample buffer. Add 1–2 μl of RNA to 18–20 μl of RNA sample buffer. Add 2–5 μl 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 for the analysis of DNA samples.

E. Synthesis of Capped RNA Transcripts

Most eukaryotic mRNAs contain a $\text{m}^7\text{G}(5')\text{ppp}(5')\text{G}$ cap at the 5'-end, which is important for the binding of translation initiation factors and contributes to mRNA stability. The use of capped RNA is suggested for programming certain translation systems (e.g., *Xenopus* oocytes). In rabbit reticulocyte- and wheat germ-based translation systems, some capped transcripts may demonstrate increased translation efficiency.

Increasingly, uncapped messages are being used effectively in reticulocyte and wheat germ systems, provided the proper concentration of the appropriate potassium salt is supplied (1). In rabbit reticulocyte lysate, potassium chloride (not potassium acetate) at levels 20mM above the maximal stimulatory level has been shown to provide the optimal conditions for the synthesis of authentic products from uncapped mRNA (9). Promega's Flexi[®] Rabbit Reticulocyte Lysate System^(c,d,h) (Cat.# L4540) provides lysate devoid of added salts or DTT and provides potassium chloride, magnesium chloride and DTT for optimization of translation of uncapped or capped messages.

The following protocol incorporates a cap analog into the transcript during the RiboMAX[™] transcription reaction. It is the same protocol as in Section IV.A, but the final GTP concentration is reduced to 0.6mM and $\text{m}^7\text{G}(5')\text{ppp}(5')\text{G}$, such as the Ribo m^7G Cap Analog (Cat.# P1711), is added to a final concentration of 3mM. Incorporating a cap analog may reduce the yield of RNA to 20–50% of the standard reaction. The ratio of cap analog:GTP is 5:1 in the following protocol, but can be varied from 10:1 to 1:1 to balance the percentage of capped products with the efficiency of the transcription reaction. Higher yields of longer capped transcripts may be optimized by increasing the concentration of GTP in the following protocol. Higher yields of smaller capped transcripts may be obtained by increasing the incubation time, the amount of RNA polymerase and the concentration of template DNA.

Synthesis of Capped RNA Transcript in vitro

1. Synthesize RNA in vitro using the following reaction mix:

SP6 Reaction Components	Sample Reaction
SP6 Transcription 5X Buffer	20µl
rNTPs (25mM ATP, CTP, UTP and 3mM GTP)	20µl
linear DNA template (5–10µg total) plus Nuclease-Free Water	42.5µl
Ribo m ⁷ G Cap Analog, 40mM	7.5µl
Enzyme Mix (SP6)	10µl
final volume	100µl
T7 Reaction Components	Sample Reaction
T7 Transcription 5X Buffer	20µl
rNTPs (25mM ATP, CTP, UTP and 2mM GTP)	30µl
linear DNA template (5–10µg total) plus Nuclease-Free Water	32.5µl
Ribo m ⁷ G Cap Analog, 40mM	7.5µl
Enzyme Mix (T7)	10µl
final volume	100µl

Larger-scale reactions may be performed by increasing the volumes proportionally.

2. Gently pipet the reaction to mix and incubate at 37°C for 2–4 hours.
3. Remove DNA template as described in Section IV. B.

V. Troubleshooting

Symptoms	Possible Causes	Comments
Low amounts of RNA synthesized using standard transcription protocol	Precipitation of the DNA template by the spermidine in the Transcription 5X Buffer	Make sure the components of the reaction are assembled at room temperature and in the order listed.
	NaCl concentration is too high (>30mM)	Residual NaCl used to precipitate the template DNA may inhibit the RNA polymerase activity by as much as 50%. The template DNA may be desalted by column chromatography and reprecipitated in the presence of another salt. Wash the resulting pellet 1–2 times with 70% ethanol.
	RNase contamination	The use of Recombinant RNasin® Ribonuclease Inhibitor is recommended for all in vitro transcription reactions. Any solutions not provided should be made up in water that has been treated with 0.1% DEPC. Individual transcription components may be purchased directly from Promega.

Note: Free cap analog can inhibit processes such as translation. Unincorporated cap analog should be removed by isopropanol precipitation (Section IV.B.) or chromatography (Section IV.C.).

Note: Optimize the capping reaction by adjusting the cap analog:GTP ratio between 10:1 and 1:1 to balance the percentage of capped products with the efficiency of the transcription reaction. Increasing the GTP concentration increases the yields of long transcripts.

For questions not addressed here, please contact your local Promega Branch Office or distributor. Contact information available at: www.promega.com.

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V. Troubleshooting (continued)

Symptoms	Possible Causes	Comments
Low amounts of RNA synthesized using standard transcription protocol (continued)	Inactive RNA polymerase	The activity of the individual RNA polymerase may be evaluated by in vitro transcription of the control template or supercoiled plasmid containing the appropriate RNA polymerase promoter.
Presence of incomplete transcripts	Premature termination of RNA synthesis	Subclone the sequence of interest into a different vector in which transcription is initiated by a different RNA polymerase. Some sequences recognized as terminators by one RNA polymerase are not recognized as efficiently by another. Lower the temperature of incubation from 37°C to 30°C. This has been shown to increase the proportion of full-length transcripts in some cases (10).
Presence of transcripts larger than expected	Protruding 3' termini on the DNA template	If the DNA template has been linearized with a restriction enzyme that generates a protruding 3'-terminus, transcription results in the synthesis of significant amounts of long RNA molecules that are initiated at the terminus of the template (7). If it is impossible to avoid using a restriction enzyme of this type, the linear DNA should be "blunt-ended" with DNA Polymerase I Large (Klenow) Fragment before use in a transcription reaction (see Section III.B).
	Nonlinearized plasmid is present in the sample	Analyze the sample by gel electrophoresis. If undigested vector is noted, redigest with the appropriate restriction enzyme.

VI. Composition of Buffers and Solutions

RNA loading buffer

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

Use a high grade glycerol. Lower grades of glycerol contain ribonuclease activity. Aliquot RNA loading buffer and store at -20°C.

RNA sample buffer

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

Dispense into aliquots and store at -20°C for up to 6 months. Do not freeze-thaw more than twice.

MOPS buffer

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

Transcription 5X Buffer (provided)

400mM HEPES-KOH (pH 7.5)
 160mM MgCl₂ (for SP6)
 120mM MgCl₂ (for T7)
 10mM spermidine
 200mM DTT

TE buffer

10mM Tris-HCl (pH 8.0)
 1mM EDTA

TE-saturated phenol:chloroform:isoamyl alcohol (25:24:1)

Mix equal parts of TE buffer and phenol and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform:isoamyl alcohol (24:1).

Note: For removal of the DNA template following transcription (Section IV.B), use TE buffer at pH 4.5 rather than TE buffer at pH 8.0.

VII. Related Products
Related Systems

Product	Size	Cat.#
Riboprobe® System – SP6(b,d)*		P1420
Riboprobe® System – T3(b,d)*		P1430
Riboprobe® System – T7(b,d)*		P1440
Riboprobe® System Buffers*		P1121
Ribo m ⁷ G Cap Analog	10 A ₂₅₄ units	P1711
	25 A ₂₅₄ units	P1712

*For Laboratory Use.

DNA Purification Products

Product	Size	Cat.#
Wizard® Plus SV Minipreps DNA Purification System(l)	50 preps	A1330
Wizard® Plus Minipreps DNA Purification System(e)	50 preps	A7100
	100 preps	A7500
	250 preps	A7510
Wizard® Plus Midipreps DNA Purification System(e)	25 preps	A7640
Wizard® Plus Maxipreps DNA Purification System(e)	10 preps	A7270
Wizard® Plus Megapreps DNA Purification System(e)	5 preps	A7300
Wizard® DNA Clean-Up System(e)	100 preps	A7280
Wizard® PCR Preps DNA Purification System(g)	50 preps	A7170

For Laboratory Use.

Single-Stranded RNA Markers

Product	Size	Cat.#
RNA Markers, 0.28–6.58kb ^(d)	50µl	G3191

For Laboratory Use.

Luciferase Assay System

Product	Size	Cat.#
Luciferase Assay System ^(h,i)	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer ^(h,i)	100 assays	E4030
Luciferase Assay Reagent ^(h,i)	1,000 assays	E1483

Translation Systems

Product	Size	Cat.#
Flexi [®] Rabbit Reticulocyte Lysate ^(c,d,h)	1ml	L4540
Rabbit Reticulocyte Lysate, Nuclease Treated ^{(c,d,h)*}	1ml	L4960
Wheat Germ Extract	1ml	L4380
Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination System		L4330

*For Laboratory Use.

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(a) The RiboMAX™ Large Scale RNA Production System–T7 (Cat.# P1300) is covered by U.S. Pat. No. 5,256,555 and is sold under a license from Ambion, Inc. It is intended for research use only. Parties wishing to use this product for other applications should contact Ambion, Inc.

(b) U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. No. 3009458 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

(c) The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(d) U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

(e) U.S. Pat. Nos. 5,658,548, 5,808,041 and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures. Other patents are pending.

(f) The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

(g) Licensed under U.S. Pat. No. 5,075,430.

(h) U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289, 5,814,471, Australian Pat. No. 649289 and European Pat. No. 0 553 234 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

(i) Certain applications of this product may require licenses from others.

(j) U.S. Pat. No. 5,981,235 has been issued to Promega Corporation for methods for isolating nucleic acids using alkaline protease. Other patents are pending.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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