RiboMAX™ Large Scale RNA Production Systems

INSTRUCTIONS FOR USE OF PRODUCTS P1280 AND P1300.

Quick PROTOCOL

Transcription Protocol

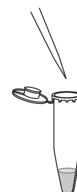
Before You Begin

- Prepare solution of chloroform:isoamyl alcohol (24:1)
- Prepare TE-saturated (pH 8.0) phenol:chloroform:isoamyl alcohol (25:24:1)
- Prepare solutions of 70% and 95% ethanol
- Prepare RNA loading buffer
- Prepare RNA sample buffer
- Prepare MOPS buffer
- Prepare 25mM rNTPs

Linearize DNA template by restriction digestion. Clean up linearized template.

Preparation and Linearization of Template

DNA templates should be linearized by digestion with the appropriate restriction endonuclease followed by a cleanup procedure, such as the Wizard® DNA Clean-Up System or phenol extraction followed by ethanol precipitation.



Assemble the reaction components appropriate for SP6 or T7 RNA Polymerase.

Transcription Procedure

1. Assemble the reaction components appropriate for SP6 or T7 RNA Polymerase at room temperature in a 1.5ml microcentrifuge tube. After all the components are added, mix by pipetting gently.

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

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

Larger scale reactions may be performed by increasing ALL volumes proportionally.

Incubate at 37°C for 2–4 hours.

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2. Pipet gently and incubate at 37°C for 2–4 hours.

DNA templates can be removed by DNase treatment. RNA can be visualized by gel electrophoresis.

See additional protocol information in Technical Bulletin #TB166, available online at **www.promega.com/tbs**

ORDERING/TECHNICAL INFORMATION:
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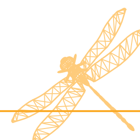

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Printed in USA. Revised 12/12.
Part# 9FB059
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