Riboprobe® in vitro Transcription Systems

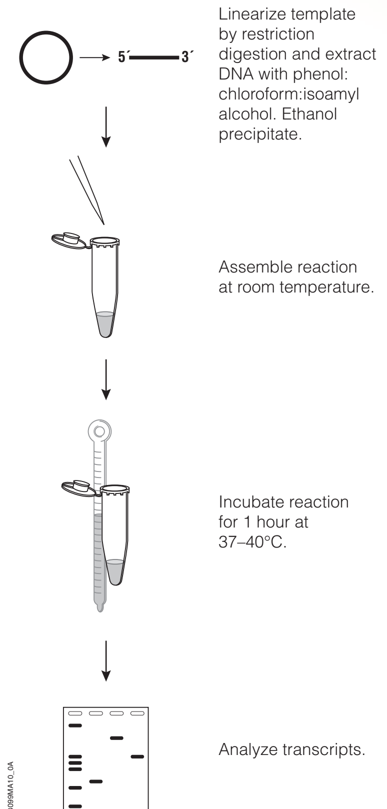
INSTRUCTIONS FOR USE OF PRODUCTS P1420, P1430, P1440, P1450 AND P1460.

Quick PROTOCOL

Transcription Protocol

Template Preparation

Perform restriction digestion, and then extract the DNA with phenol:chloroform:isoamyl alcohol. Ethanol precipitate and resuspend in TE or water.



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Transcription Protocol

1. Add the following components at room temperature in the order listed:

Component	Amount
Transcription Optimized 5X Buffer	4µl
DTT, 100mM	2µl
Recombinant RNasin® Ribonuclease Inhibitor	20–40u
rATP, rGTP and rUTP (2.5mM each) (prepared by mixing 1 volume deionized water with 1 volume of each of the 10mM rATP, rGTP and rUTP stocks supplied)	4µl
100µM rCTP (diluted from stock)	2.4µl
linearized template DNA (0.2–1.0mg/ml in water or TE)	1µl
[α-^{32}P]rCTP (50µCi at 10µCi/µl)	5µl
SP6, T3 or T7 RNA Polymerase	15–20u
final volume	20µl

2. Incubate for 1 hour at 37–40°C.
3. Remove 1µl from this reaction to determine the percent incorporation and specific activity of the probe.

Positive Control Protocol

1. Add the following components at room temperature in the order listed:

Component	Amount
Transcription Optimized 5X Buffer	4µl
DTT, 100mM	2µl
Recombinant RNasin® Ribonuclease Inhibitor	20–40u
rATP, rCTP, rGTP and rUTP (2.5mM each) (prepared by mixing 1 volume of each of the 10mM rNTP stocks supplied)	4µl
pGEM® Express Positive Control Template	1µg
SP6, T3 or T7 RNA Polymerase	15–20u
Nuclease-Free Water to a final volume of	20µl

2. Incubate for 1 hour at 37–40°C.

Analysis of Transcripts (Optional)

Add 5µl of the control transcription reaction to 15µl of sample buffer. Add 2–5µl of RNA loading buffer and heat the sample for 5–10 minutes at 65–70°C prior to loading onto the gel. Run the gel under standard conditions for analysis of RNA samples. A small aliquot of the radioactive transcript can also be run on a gel and analyzed by autoradiography or phosphorimaging instrumentation.

ORDERING/TECHNICAL INFORMATION:
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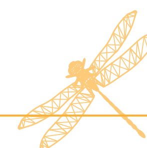

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Transcription Protocol (continued)

Removal of Template DNA

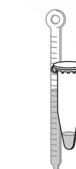
1. Add RQ1 RNase-Free DNase (1u/µg DNA). Incubate 15 minutes at 37°C.
2. Extract with 1 volume of citrate-saturated phenol:chloroform:isoamyl alcohol (25:24:1 [pH 4.5]). Vortex for 1 minute and centrifuge (12,000 × *g*) for 2 minutes.
3. Transfer the upper, aqueous phase to a fresh tube and add 1 volume of chloroform:isoamyl alcohol (24:1). Vortex for 1 minute and centrifuge (12,000 × *g*) for 2 minutes.
4. Transfer the upper, aqueous phase to a fresh tube. Add 0.5 volume of 7.5M ammonium acetate and 2.5 volumes of 100% ethanol. Mix and place at –70°C for 30 minutes. Centrifuge for 20 minutes.
5. Remove the supernatant and wash the pellet with 1ml of 70% ethanol. Dry the pellet under vacuum. If removal of unincorporated nucleotides is desired, see Section IV.E of TM016. Suspend the RNA in 10–20µl of TE buffer or water and store at –70°C.

Capping RNA for In Vitro Translation

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

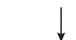
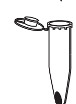
Component	Amount
Transcription Optimized 5X Buffer	10µl
DTT, 100mM	5µl
Recombinant RNasin® Ribonuclease Inhibitor	50u
nucleotides (2.5µl each of 10mM rATP, rCTP, rUTP and 1mM rGTP)	10µl
Ribo m^{7}G Cap Analog, 5mM	5µl
linearized template DNA (1mg/ml in water or TE buffer)	5µl
SP6, T3 or T7 RNA Polymerase	40u
Nuclease-Free Water to a final volume of	50µl

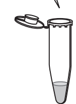
2. Incubate for 1 hour at 37°C. To increase the RNA yield, add an additional 40 units of RNA polymerase and incubate for 1 hour longer.
3. Proceed with DNase I treatment to remove the template, followed by purification of the RNA as described above and in Section 4.D of TM016.


Removal of Template DNA

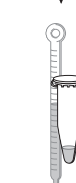
Add RQ1 DNase and incubate 15 minutes at 37°C.

Extract with citrate-saturated phenol:chloroform: isoamyl alcohol, followed by chloroform:isoamyl alcohol extraction.



Precipitate RNA. Wash and dry pellet. Suspend RNA in TE buffer or water and store at –70°C.


Capping RNA for in vitro Translation

Synthesize RNA by adding reaction components.


Incubate for 1 hour at 37°C. Proceed with DNase I treatment and purification.

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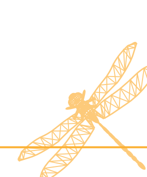
See additional protocol information in Technical Manual #TM016, available online at: **www.promega.com/tbs**



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