USER GUIDE

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by life technologies™

MEGAscript® Kit

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MEGAscript® Kit

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Product overview

Product description

MEGAscript® Kits are ultra-high yield in vitro transcription kits. The high yields are achieved by modifying typical transcription reaction conditions so that very high nucleotide concentrations can be effectively used. The MEGAscript® Kits contain in vitro transcription reaction components for twenty-five or forty 20 µL reactions and a control template. Each kit will yield a total of 3–5 mg of RNA (approximately 100 µg of RNA or more per reaction) from the control template supplied with the kit. This corresponds to 400–650 moles of RNA for each mole of template. Smaller templates typically yield a lower mass and a higher molar yield of product.

MEGAscript® Kits are intended for the synthesis of large amounts of unlabeled or low specific activity RNA for a variety of uses including in vitro translation, antisense/microinjection studies, and isolation of RNA binding proteins. In large-scale transcription reactions, the concentration of all 4 nucleotides is high, well above the K_m for the enzyme. MEGAscript® Kits typically yield over 10 times more RNA than conventional in vitro transcription reactions (Krieg and Melton, 1987). MEGAscript® Kits are not recommended for synthesis of high specific activity probes.

Materials provided with the kit

The MEGAscript® Kit should be stored in a non-frost-free freezer. Keep all reagents on ice while using the kit; the nucleotides and enzymes are especially labile.

Components specific to the RNA polymerase in the kit

The SP6, T7, or T3 Enzyme Mix and the 10X Reaction Buffer are specifically calibrated for each lot and RNA polymerase. Mixing components from different lots, or from kits for different RNA polymerases (SP6, T7, T3) will compromise RNA yield.

Cat. no. AM1333 (25 rxn)	All 40 rxn kits	Component	Storage
50 µL	80 µL	Enzyme Mix (SP6, T7, or T3)	–20°C
50 µL	80 µL	10X Reaction Buffer† (SP6, T7, or T3)	–20°C
50 µL	80 µL	ATP Solution‡ (SP6, T7, or T3)	–20°C
50 µL	80 µL	CTP Solution (SP6, T7, or T3)	–20°C
50 µL	80 µL	GTP Solution (SP6, T7, or T3)	–20°C
50 µL	80 µL	UTP Solution (SP6, T7, or T3)	–20°C

† Salts, buffer, dithiothreitol, and other ingredients

‡ The ATP, CTP, GTP, and UTP Solutions are supplied at 75 mM for T7 and T3 kits, or at 50 mM for SP6 kits.

All MEGAscript® Kits include the following components:

Amount	Component	Storage
1.75 mL	Nuclease-free Water	any temp†
100 µL	TURBO DNase (2 U/µL)	–20°C
10 µL	pTRI-Xef, 0.5 mg/mL (Control Template)	–20°C
1 mL	Ammonium Acetate Stop Solution 5 M ammonium acetate, 100 mM EDTA	–20°C
1.4 mL	Lithium Chloride Precipitation Solution 7.5 M lithium chloride, 50 mM EDTA	–20°C
1.4 mL	Gel Loading Buffer II 1–2X gel loading solution for TBE polyacrylamide and agarose gels containing: 95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 18 mM EDTA, and 0.025% SDS	–20°C

† Store Nuclease-free Water at –20°C, 4°C or room temp.

Materials not provided with the kit

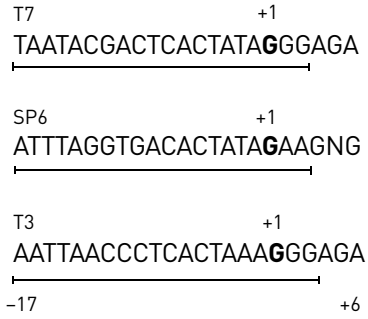
- DNA template: The DNA template must have the correct RNA polymerase promoter site (T7, T3, or SP6) upstream of the sequence to be transcribed. The suggested template concentration is 0.5 µg/µL in water or TE (10 mM Tris-HCl (pH 7–8), 1 mM EDTA).
- (optional) Labeled nucleotide(s): Any [α-^{32}P] labeled nucleotide can be added to the reaction as a tracer to facilitate quantitation of the RNA synthesized. Any specific activity is acceptable.
- (optional) For purification of the synthesized RNA:
  - Buffer- or water-saturated phenol/chloroform
  - Isopropanol
  - Spin Columns

MEGAscript® Kit procedure

Preparation of template DNA

Linearized plasmid DNA, and PCR products that contain an RNA polymerase promoter site can be used as templates for in vitro transcription with the MEGAscript® Kit. In general, any DNA with a promoter site, that is pure enough to be easily digested with restriction enzymes can be used for in vitro transcription.

Figure 1 Phage Polymerase Promoters: Minimal Sequence Requirements



The +1 base (in bold) is the first base incorporated into RNA during transcription. The underline shows the minimum promoter sequence needed for efficient transcription.

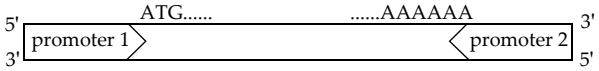
Template size

The MEGAscript® Kit is designed to function best with templates that code for RNA transcripts of about ***0.5 kb and longer***. The kit can be used to produce shorter RNA, but modify the reaction as described in section "Optimizing yield of short transcripts" on page 24.

Orientation

If ***sense RNA*** is needed, it is important to transcribe using the RNA polymerase corresponding to the phage promoter at the 5', or amino-terminal side of the coding region of the protein (using promoter 1 in the diagram below). If the template consists of a plasmid, it should be linearized in the polylinker at the opposite (3' or carboxy-terminal side) of the protein-coding region.

Antisense (mRNA-complementary) transcripts will be synthesized if the RNA polymerase corresponding to the RNA phage promoter at the 3', or carboxy-terminal side of the coding region of the protein is used (using promoter 2 in the diagram below).



Transcription using the RNA polymerase corresponding to promoter 1 will make sense RNA (the same sequence as the mRNA). If the RNA polymerase for promoter 2 is used, antisense RNA will be transcribed.

Plasmid templates

DNA should be relatively free of contaminating proteins and RNA. We observe the greatest yields with very clean template preparations. Most commercially available plasmid preparation systems yield DNA that works well in the MEGAscript® Kit.

Linearization

Plasmid DNA must be linearized with a restriction enzyme downstream of the insert to be transcribed. Circular plasmid templates will generate extremely long, heterogeneous RNA transcripts because RNA polymerases are very processive. It is generally worthwhile to examine the linearized template DNA on a gel to confirm that cleavage is complete. Since initiation of transcription is one of the limiting steps of in vitro transcription reactions, even a small amount of circular plasmid in a template prep will generate a large proportion of transcript.

Although we routinely use all types of restriction enzymes, there has been one report of low level transcription from the inappropriate template strand in plasmids cut with restriction enzymes leaving 3' overhanging ends (produced by *Kpn* I, *Pst* I, etc.; Schendorn and Mierindorf, 1985).

After linearization

Terminate the restriction digest by adding the following:

- 1/20th volume 0.5 M EDTA
- 1/10th volume of 3 M Na acetate ***or*** 5 M NH_4 acetate
- 2 volumes of ethanol

Mix well and chill at –20°C for at least 15 min. Then pellet the DNA for 15 min in a microcentrifuge at top speed. Remove the supernatant, re-spin the tube for a few seconds, and remove the residual fluid with a very fine-tipped pipet. Resuspend in dH2O or TE buffer at a concentration of 0.5–1 µg/µL.

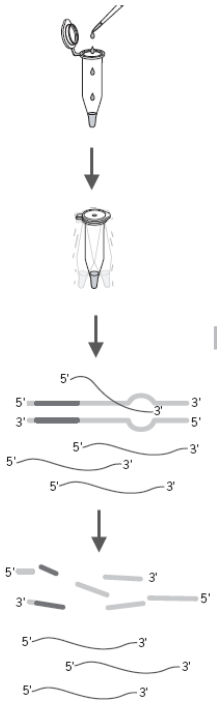
Proteinase K treatment

Note that DNA from some miniprep procedures may be contaminated with residual RNase A. Also, restriction enzymes occasionally introduce RNase or other inhibitors of transcription. When transcription from a template is suboptimal, it is often helpful to treat the template DNA with proteinase K (100–200 µg/mL) and 0.5% SDS for 30 min at 50°C, follow this with phenol/chloroform extraction (using an equal volume) and ethanol precipitation.

PCR templates

DNA generated by PCR can be transcribed directly from the PCR provided it contains an RNA Polymerase promoter upstream of the sequence to be transcribed. PCR products should be examined on an agarose gel before use as a template in MEGAscript® to estimate concentration, and to verify that the products are unique and the expected size.

Procedure overview



Assemble

1. Thaw the frozen reagents (page 9)
2. Assemble transcription reaction at room temp (page 9)

Mix

3. Mix thoroughly (page 10)

Incubate

4. Incubate at 37°C, 2–4 hr (page 10)

DNase Treatment

5. (optional) Add 1 µL TURBO DNase, mix well and incubate 15 min at 37°C (page 10)

Transcription reaction assembly

1. Thaw the frozen reagents

   Place the RNA Polymerase Enzyme Mix on ice, it is stored in glycerol and will not be frozen at –20°C.

   Vortex the 10X Reaction Buffer and the 4 ribonucleotide solutions (ATP, CTP, GTP, and UTP) until they are completely in solution. Once thawed, store the ribonucleotides on ice, but ***keep the 10X Reaction Buffer at room temperature while assembling the reaction***.

   All reagents should be microfuged briefly before opening to prevent loss and/or contamination of material that may be present around the rim of the tube.

2. Assemble transcription reaction at room temp

   The spermidine in the 10X Reaction Buffer can coprecipitate the template DNA if the reaction is assembled on ice.

   Add the 10X Reaction Buffer after the water and the ribonucleotides are already in the tube.

   The following amounts are for a single 20 µL reaction. Reactions may be scaled up or down if desired.

IMPORTANT! The following reaction setup is recommended when the RNA produced will be ≥0.5 kb in length. For transcripts shorter than this, consider the suggestions in section "Optimizing yield of short transcripts" on page 24.

Note: For convenience, mix equal volumes of the four ribonucleotide solutions together and add 8 µL of the mixture to a standard 20 µL reaction instead of adding the ribonucleotides separately.

Amount	Component
to 20 µL	Nuclease-free Water
2 µL	ATP solution
2 µL	CTP solution
2 µL	GTP solution
2 µL	UTP solution
2 µL	10X Reaction Buffer
(1 µL)	(optional) [α-^{32}P]UTP as a tracer
0.1–1 µg	linear template DNA†
2 µL	Enzyme Mix

† Use 0.1–0.2 µg PCR-product template or ~1 µg linearized plasmid template.

3. Mix thoroughly

   Gently flick the tube or pipette the mixture up and down gently, and then microfuge tube briefly to collect the reaction mixture at the bottom of the tube.

4. Incubate at 37°C, 2–4 hr

   The first time a new template is transcribed, the recommended incubation time is 2–4 hours. The optimal incubation time for a given template will vary depending on the size and transcriptional efficiency of your template. For short transcripts (less than 500 nt), a longer incubation time (up to ~16 hours) may be advantageous, since more transcription initiation events are required to synthesize a given mass amount of RNA, compared to transcription of longer templates. (See section “Optimizing yield of short transcripts” on page 24 for more details.)

   To determine the optimum incubation time for maximum yield with a given template, a time-course experiment can be done. To do this, set up a MEGAscript® reaction, and remove aliquots of the reaction at various intervals (for example after 1, 2, 4, or 6 hr, and overnight incubation). Assess results by TCA precipitation or other means (see section “Quantitation of reaction products” on page 12.)

   If the reaction is trace-labeled:

   After the incubation (before or after TURBO DNase treatment), remove an aliquot of trace-radiolabeled reactions to assess yield by TCA precipitation (see step on page 13).

5. (optional) Add 1 µL TURBO DNase, mix well and incubate 15 min at 37°C

   This DNase treatment removes the template DNA. For many applications it may not be necessary because the template DNA will be present at a very low concentration relative to the RNA.

   a. Add 1 µL TURBO DNase, and mix well (the reaction may be viscous).

   b. Incubate at 37°C for 15 min.

Recovery of the RNA

The degree of purification required after the transcription reaction depends on what will be done with the RNA. Four different methods follow, choose one or more according to your application and resources.

MEGAclear™ Kit

The MEGAclear™ Kit was developed specifically for purifying RNA from high yield in vitro transcription reactions. The quick and simple procedure removes nucleotides, short oligonucleotides, proteins, and salts from RNA. The RNA recovered can be used for any application that requires high purity RNA.

Lithium chloride precipitation

Lithium Chloride (LiCl) precipitation is a convenient and effective way to remove unincorporated nucleotides and most proteins. Lithium chloride precipitation, however, does not precipitate transfer RNA and may not efficiently precipitate RNAs smaller than 300 nucleotides. Also, the concentration of RNA should be at least 0.1 µg/µL to assure efficient precipitation. To precipitate from MEGAscript® reactions that are thought to have very low yields of RNA, do not dilute the transcription reaction with water prior to adding the LiCl Precipitation Solution in the step below.

1. Stop the reaction and precipitate the RNA by adding 30 µL Nuclease-free Water and 30 µL LiCl Precipitation Solution.
2. Mix thoroughly. Chill for ≥30 min at –20°C.
3. Centrifuge at 4°C for 15 min at maximum speed to pellet the RNA.
4. Carefully remove the supernatant. Wash the pellet once with ~1 mL 70% ethanol, and re-centrifuge to maximize removal of unincorporated nucleotides.
5. Carefully remove the 70% ethanol, and resuspend the RNA in a solution or buffer† appropriate for your application. Determine the RNA concentration and store frozen at –20°C or –70°C.

Spin column chromatography

Spin columns will remove unincorporated nucleotides.

Prepared spin columns such as NucAway™ Spin Columns can be used by following the manufacturer's instructions. Alternatively, instructions for preparing spin columns are given in section "Spin column preparation and use" on page 29.

Phenol:chloroform extraction and isopropanol precipitation

This is the most rigorous method for purifying transcripts. It will remove all enzyme and most of the free nucleotides from MEGAscript® Kit reactions. Since the RNA is precipitated, this method can also be used for buffer exchange.

1. Add 115 µL Nuclease-free Water and 15 µL Ammonium Acetate Stop Solution, and mix thoroughly.

† Life Technologies offers several products for RNA storage; these include:
Nuclease-free Water (not DEPC-treated): Cat. no. AM9930–AM9939
THE RNA Storage Solution: Cat. no. AM7000, AM7001
TE Buffer: Cat. no. AM9860, AM9861
0.1 mM EDTA: Cat. no. AM9912

2. Extract with an equal volume of phenol/chloroform (it can be water-saturated, buffer-saturated, or acidic), and then with an equal volume of chloroform. Recover aqueous phase and transfer to new tube.
3. Precipitate the RNA by adding 1 volume of isopropanol and mixing well.
4. Chill the mixture for at least 15 min at –20°C. Centrifuge at 4°C for 15 min at maximum speed to pellet the RNA. Carefully remove the supernatant solution and resuspend the RNA in a solution or buffer† appropriate for your application.
5. Store frozen at –20°C or –70°C.

Quantitation of reaction products

Quantitation by UV light absorbance

Reading the A_{260} of a diluted aliquot of the reaction is clearly the simplest way to determine yield, but any unincorporated nucleotides and/or template DNA in the mixture will contribute to the reading. Typically, a 1:300 dilution of an aliquot of a MEGAscript® reaction will give an absorbance reading in the linear range of a spectrophotometer.

For single-stranded RNA, 1 A_{260} unit corresponds to 40 µg/mL, so the RNA yield can be calculated as follows:

$$A_{260} \times \text{dilution factor} \times 40 = \mu g/mL \text{ RNA}$$

Assessing RNA yield with RiboGreen®

If you have a fluorometer, or a fluorescence microplate reader, Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using RiboGreen®.

Quantitation by ethidium bromide fluorescence

The intensity of ethidium bromide staining can be used to get a rough estimation of the RNA yield.

Ethidium bromide spot assay

If unincorporated nucleotides have been removed, an ethidium bromide spot assay can be used to quantitate RNA concentration. Make a standard curve with several 2-fold dilutions of an RNA solution of known concentration. Start at about 80 ng/µL, and go down to about 1.25 ng/µL. Make a few dilutions of the unknown RNA, and add ethidium bromide to 1 ng/µL to each dilution of both RNAs. Spot 2 µL of the standard curve RNA samples and the unknown RNA dilutions onto plastic wrap placed on a UV transilluminator. Compare the fluorescence of the RNAs to estimate the concentration of the unknown RNA sample. Make sure that the sample dilutions are in the linear range of ethidium bromide fluorescence. This assay will detect as little as 5 ng of RNA with about a 2-fold error.

Denaturing gel electrophoresis

If unincorporated nucleotides have not been removed from the reaction, an aliquot of the MEGAscript® reaction should be run on a denaturing agarose or acrylamide gel alongside an aliquot of an RNA of known concentration. See section "Additional Procedures" on page 23 for instructions on running gels. Stain the samples with ethidium bromide, and simply compare the intensity of the unknown sample to the known RNA to estimate its concentration.

Agilent® bioanalyzer and RNA LabChip® Kits

RNA can be evaluated on an Agilent® 2100 bioanalyzer using one of their RNA LabChip® Kits to get an idea of what percentage of the transcription products are full-length. Follow the manufacturer's instructions for using the bioanalyzer and the RNA LabChip® Kit.

Quantitation by trace radiolabeling

1. TCA precipitation

   If a trace amount of radiolabel was included in the MEGAscript® reaction, it can be used to determine yield. First precipitate with TCA to determine the proportion of radiolabel that was incorporated into RNA. (TCA will precipitate nucleic acids as small as 18 nt.)

   a. Dilute 1 µL of the completed MEGAscript® reaction into 150 µL of carrier DNA or RNA (1 mg/mL), and mix thoroughly. Sheared Salmon Sperm DNA (Cat. no. AM9680) can be used for this purpose.

   b. Transfer 50 µL of the RNA + carrier nucleic acid mixture to aqueous scintillation cocktail and count in a scintillation counter. This will measure the total amount of radiolabel present in the reaction mixture (unincorporated and incorporated counts).

   c. Transfer another 50 µL of the RNA + carrier nucleic acid mixture to a 12 x 75 mm glass tube, and add 2 mL of cold 10% TCA (trichloroacetic acid). Mix thoroughly and place on ice for 10 min. This will precipitate nucleic acids, but not free nucleotides.

   d. Collect the precipitate via vacuum filtration through a Whatman GF/C glass fiber filter (or its equivalent).

   e. Rinse the tube twice with 1 mL of 10% TCA and then rinse once with 3–5 mL of 95% ethanol. Pass each of the rinses through the GF/C filter.

   f. Place the filter in a scintillation vial, add aqueous scintillation cocktail, and count in a scintillation counter. This will give the TCA precipitated counts (radiolabel that was incorporated into RNA).

   g. Divide the cpm in the above step by the cpm in step b. to determine the fraction of label incorporated into RNA (multiply by 100 for percent incorporation).

$$\frac{\text{TCA ppt cpm}}{\text{Total cpm}} \times 100 = \%\ \text{incorporation}$$

2. Calculation of yield

   Once the percent incorporation of radiolabel is known, it can be used to calculate the mass yield of RNA transcribed in the MEGAscript® reaction. Each 1% incorporation corresponds to about 2 µg of RNA synthesized in a T7 or T3 reaction. For SP6 Kits, each 1% incorporation corresponds to about 1.3 µg of RNA synthesized.

   In a T7 or T3 reaction, if all four nucleotides are incorporated equally, 198 µg of RNA will be produced if all of the 7.5 mM of NTP is incorporated into RNA (the sum of the molecular masses of the 4 nucleotides in RNA is about 1320).

$$\frac{7.5\ \text{mM}}{10^6\ \mu\text{L}} \times \frac{1320\ \text{g}}{1000\ \text{mM}} \times 20\ \mu\text{L} = \frac{1.98 \times 10^5\ \text{g}}{10^9} = 1.98 \times 10^{-4}\ \text{g} = 198\ \mu\text{g}$$

The standard SP6 MEGAscript® reaction contains 5 mM of each NTP, so when that value is substituted in the above equation the maximum theoretical yield for SP6 MEGAscript® reactions is 132 µg.

Troubleshooting

Use of the Control Template

The pTRI-Xef control template is a linearized TRIPLEscript plasmid containing the 1.85 kb Xenopus elongation factor 1α gene under the transcriptional control of tandem SP6, T7, and T3 promoters (pTRI-Xef 1). Any one of the three RNA polymerases can be used to synthesize the control RNA. When transcribed with the following RNA polymerases, sense transcripts of the indicated length are produced from this template:

Enzyme	Transcript Size
SP6	1.92 kb
T7	1.89 kb
T3	1.86 kb

These transcripts will produce a 50.2 kD protein when translated.

1. Reaction setup

   Use 2 µL (1 µg) of pTRI-Xef in a standard MEGAscript® reaction as described in section "Transcription reaction assembly" on page 9.

2. Expected yield from the control reaction

   The yield from the control reaction for T7 and T3 Kits should be 80–100 µg of RNA, and 50–80 µg with the SP6 Kits. If a [^{32}P]NTP was added to the transcription reaction as a tracer, approximately 30–40% of the radiolabel should be incorporated.

3. What to do if the control reaction doesn't work as expected

   If the yield of RNA from the control reaction is low, something may be wrong either with the procedure or the kit, or the quantitation is in error.

   a. Double check the RNA quantitation

      To confirm that the quantitation is correct, verify the yield by an independent method. For example if TCA precipitation was used to assess yield, try also running an aliquot of the reaction on an agarose gel.

   b. Try the positive control reaction again

      If the yield is indeed low by two different measurements, there may be a technical problem with the way the kit is being used. For example, the spermidine in the 10*X* Reaction Buffer may cause precipitation of the template DNA if it is not diluted by the other ingredients prior to adding the DNA. (This is the reason that the water is added first.) Repeat the reaction, following the procedure carefully. If things still don't go well, contact Technical Services for more ideas.

Troubleshooting low yield

The amount of RNA synthesized in a standard 20 µL MEGAscript® reaction should be 50 µg and may exceed 100 µg; however, there is a great deal of variation in yield from different templates. If the yield is low, the first step in troubleshooting the reaction is to use the pTRI-Xef control template in a standard MEGAscript® reaction.

1. Neither my template nor the control reaction works

   Double check that you have followed the procedure accurately, and consider trying the control reaction a second time. If the kit control still doesn't work, it is an indication that something may be wrong with the kit, call our Technical Support group for more ideas.

2. The control reaction works, but my template gives low yield

   If the transcription reaction with your template generates full-length, intact RNA, but the reaction yield is significantly lower than the amount of RNA obtained with the pTRI-Xef control template, it is possible that contaminants in the DNA are inhibiting the RNA polymerase. A mixing experiment can help to differentiate between problems caused by inhibitors of transcription and problems caused by the sequence of a template. Include three reactions in the mixing experiment, using the following DNA templates:

   1. 1 µL pTRI-Xef control template
   2. experimental DNA template (0.5 µg plasmid or 2–6 µL PCR product)
   3. a mixture of 1 and 2

   Assess the results of the mixing experiment by running 2–4 µL of a 1:5 dilution of each transcription reaction on a denaturing gel as described in section "Analysis of transcription products by gel electrophoresis" on page 23.

   a. Transcription of the control template is inhibited by the presence of your template. (See Figure 2.A)

      This implies that inhibitors are present in your DNA template. Typical inhibitors include residual SDS, salts, EDTA, and RNases. Proteinase K treatment frequently improves template quality.

      Treat template DNA with Proteinase K (100–200 µg/mL) and SDS (0.5%) for 30 min at 50°C, followed by phenol/chloroform extraction and ethanol precipitation. Carry-over of SDS can be minimized by diluting the nucleic acid several fold before ethanol precipitation, and excess salts and EDTA can be removed by vigorously rinsing nucleic acid pellets with cold 70% ethanol before resuspension.

   b. Adding your template to the reaction with the control template does not inhibit synthesis of the control RNA. (See Figure 2.B)

This result indicates that the problem may be inherent to your template.

i. Use a different polymerase for transcription if possible

   Templates differ in transcription efficiency depending on the initiation efficiency of their promoter, the presence of internal termination signals, and their length. If the problem is due to the first or second of these issues, changing the RNA polymerase promoter used to transcribe the fragment may alleviate the problem.

ii. Check the amount and quality of template

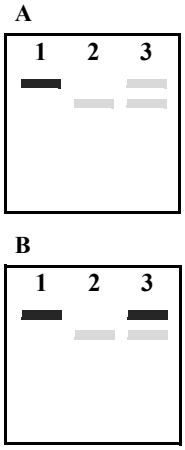
   Another possibility is that the template quantitation is inaccurate. If quantitation was based on UV absorbance and the DNA prep had substantial amounts of RNA or chromosomal DNA, the amount of template DNA may be substantially less than the calculated value.

   Also, check an aliquot of the template DNA on an agarose gel to make sure it is intact and that it is the expected size.

iii. Extend the reaction time

   Another parameter that can be adjusted is reaction time. Extending the standard 2–4 hr incubation to 6–10 hr or even overnight may improve yield.

Figure 2 Possible outcomes of mixing experiment.
1 - control template
2 - experimental template
3 - mixture of 1 and 2



Multiple reaction products, transcripts of the wrong size

Reaction products produce a smear when run on a denaturing gel

If the RNA appears degraded (e.g. smeared), remove residual RNase from the DNA template preparation before in vitro transcription. Do this by digesting the DNA prep with proteinase K (100–200 µg/mL) in the presence of 0.5% SDS for 30 min at 50°C, follow this with phenol/chloroform extraction. The RNase Inhibitor that is present in the transcription reaction, can only inactivate trace RNase contamination. Large amounts of RNase contamination will compromise the size and amount of transcription products.

Reaction products run as more than one band, or as a single band smaller than expected

1. Sample is not adequately denatured in the gel

If the amount of RNA produced is acceptable, but the size of the product is unexpected, consider that the RNA may be running aberrantly due to secondary structure. Sometimes the RNA will run as two distinct bands on a native agarose gel, but when the same RNA is run on a denaturing gel, it will migrate as a single band of the expected size.

2. Premature termination of transcription

   If denaturing gel analysis shows the presence of multiple bands or of a single band smaller than the expected size, there may be problems with premature termination by the polymerase. Possible causes of this are sequences which resemble the phage polymerase termination signals, stretches of a single nucleotides, and GC-rich templates.

   - Different phage polymerases recognize different termination signals, so using a different polymerase promoter may help.
   - Termination at single polynucleotide stretches can sometimes be alleviated by decreasing the reaction temperature (Krieg, P.A. 1990). We suggest testing 30°C, 20°C and 10°C. However, decreasing the reaction temperature will also significantly decrease the yield of the reaction.
   - There is a report that single-stranded binding (SSB) protein increased the transcription efficiency of a GC rich template (Aziz and Soreq, 1990).

Reaction products are larger than expected

1. Persistent secondary structure

   MEGAscript® products occasionally run as 2 bands; 1 larger than the expected size, and 1 at the expected size. This may occur with transcripts from the pTRI-Xef control template, even when the RNA is denatured during the electrophoresis. This phenomenon occurs because of persistent secondary structure. To verify this, the band that migrates at the expected size can be excised from the gel and run in a second denaturing gel. If the RNA runs as a doublet in the second gel also, it is a good indication that the larger band is simply an artifact of electrophoresis.

2. Circular template

   Longer-than-expected transcription products will be seen if any of the template molecules are circular. This is typically caused by incomplete digestion of a plasmid template. Since the RNA polymerases are extremely processive, even a small amount of circular template can produce a large amount of RNA.

A Supplemental Information

Related products available from Life Technologies

MEGAclear™ Cat. no. AM1908	MEGAclear™ purifies RNA from transcription, and other enzymatic reactions yielding high quality RNA free of unincorporated nucleotides, enzymes and buffer components with close to 100% recovery of input RNA.
mMESSAGE mMACHINE® Cat. nos. AM1340, AM1344, AM1348	High yield transcription kits for production of large amounts of capped RNA. These kits employ Life Technologies's novel, patented MEGAscript® technology, and include cap analog. Kits are available with T7, SP6, and/or T3 RNA polymerase.
mMESSAGE mMACHINE® T7 Ultra Kit Cat. nos. AM1345	The mMESSAGE mMACHINE® T7 Ultra Kit incorporates Anti-Reverse Cap Analog (ARCA) into Life Technologies's patented high yield transcription technology to generate RNA transcripts that yield much higher amounts of protein when translated in vitro or in vivo, than messages with traditional cap analog. The increased translation efficiency provided by ARCA is further enhanced by the addition of a poly(A) tail to the transcripts. Experiments comparing ARCA and ARCA/poly(A) tailed transcripts to cap analog and cap analog/poly(A) tailed transcripts show significantly higher levels of protein synthesis with ARCA capped RNA.
RNase-free Tubes & Tips	RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free.
RNase*Zap*® Cat. nos. AM9780, AM9782, AM9784	RNase Decontamination Solution. RNase*Zap*® is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNase*Zap*®.
NucAway™ Spin Columns Cat. no. AM10070	Guaranteed RNase- and DNase-free, NucAway™ Spin Columns provide a fast, efficient way to remove unincorporated nucleotides, and to effect buffer exchange after probe synthesis and other reactions.
RNA Storage Solutions	Three different choices for safe, RNase-free resuspension of RNA pellets. Choose one or more of the following: THE RNA Storage Solution, Cat. no. AM7000, AM7001 0.1 mM EDTA, Cat. no. AM9912 TE Buffer, Cat. no. AM9860, AM9861
TURBO DNA-*free*™ Kit Cat. no. AM1907	The TURBO DNA-*free*™ Kit employs Life Technologies's exclusive TURBO™ DNase (patent pending); a specifically engineered hyperactive DNase that exhibits up to 350% greater catalytic efficiency than wild type DNase I. The kit also includes a novel reagent for removing the DNase without the hassles or hazards of phenol extraction or alcohol precipitation—and without heat inactivation, which can cause RNA degradation. TURBO DNA-*free*™ is ideal for removing contaminating DNA from RNA preparations.

Electrophoresis Reagents	Life Technologies offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis.
Proteinase K Cat. no. AM2542, AM2548	Proteinase K is a non-specific serine protease commonly used in molecular biology to remove protein contaminants from nucleic acids. Life Technologies supplies Proteinase K in powder form, and as a 50% glycerol solution.
Phenols	Life Technologies offers a full line of prepared phenol solutions for most molecular biology needs. These premixed, quality-tested, saturated phenols are ready-to-use and eliminate the handling concerns associated with preparing phenol for use from solid phenol.
Cap Analog & Variants	Cap analog, $m^7G(5')ppp(5')G$, is used for the synthesis of 5' capped RNA by in vitro transcription. Cap analog is also used as a highly specific inhibitor of the initiation step of protein synthesis. Life Technologies also offers cap analog variants. All of the Cap Analog products are tested in vitro transcription, and are certified nuclease-free.
ARCA Cat. no. AM8045	7-methyl (3'-0-methyl) GpppG, anti-reverse cap analog, can be added to in vitro transcription reactions to produce capped RNA transcripts that incorporate the cap only in the correct orientation.

Quality control

Functional testing

All components are tested in a functional MEGAscript® assay as described in this procedure. A 20-µL reaction containing 1 µg of the control template DNA which codes for a ~1.9 kb transcript synthesized >90 µg of RNA after a 2 hr incubation.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

A sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Protease testing

A sample is incubated with protease substrate and analyzed by fluorescence.

B Recipes

10X TBE

TBE is generally used at 1X final concentration for preparing gels and/or for gel running buffer.

IMPORTANT! Do not treat TBE with diethylpyrocarbonate (DEPC).

Concentration	Component	for 1 L
0.9 M	Tris base	109 g
0.9 M	Boric Acid	55 g
20 mM	0.5 M EDTA solution	40 mL

Dissolve with stirring in about 850 mL nuclease-free water. Adjust the final volume to 1 L.

Alternatively, Life Technologies offers nuclease-free solutions of 10X TBE (Cat. nos. AM9863, AM9865) and ready-to-resuspend powdered 10X TBE packets (Cat. no. AM9864). Both are made from of ultrapure molecular biology grade reagents.

Denaturing acrylamide gel mix

5% acrylamide /8M urea gel

15 mL is enough gel solution for one 13 x 15 cm x 0.75 mm gel.

1. Mix the following:

for 15mL	Component
7.2 g	Urea (high quality) (Cat. no. AM9902)
1.5 mL	10X TBE
1.9 mL	40% Acrylamide (19 acryl:1 bis-acryl) (Cat. no. AM9022, AM9024)
to 15 mL	ddH_2O

2. Stir at room temperature until the urea is completely dissolved, then add:

120 µL	10% ammonium persulfate
16 µL	TEMED

3. Mix briefly after adding the last two ingredients, which will catalyze polymerization, then pour gel immediately. (It is not necessary to treat the gel mixture with diethylpyrocarbonate)

Gel set up

- Follow the manufacturers instructions for the details of attaching gels to the running apparatus.
- Use 1X TBE as the gel running buffer.
- It is very important to rinse the wells of urea-containing gels immediately before loading the samples.

Electrophoresis conditions

Gels should be run at about 20 V/cm gel length; for 13 cm long gel this will be about 250 V. Alternatively, denaturing acrylamide gels of this size can be run at ~25 mAmp, constant current.

RNase-free water

1. Add DEPC to 0.05% to double-distilled, deionized water (i.e. add 0.5 mL per liter of water).
2. Stir well, incubate several hours to overnight at 37°C or 42°C.
3. Autoclave 2 L or smaller volumes for at least 45 min. The scent of DEPC should be either not detectable or only very slightly detectable.

C

Additional Procedures

Analysis of transcription products by gel electrophoresis

Agarose or acrylamide?

The size of MEGAscript® reaction products can be assessed by running an aliquot of the reaction on an agarose or polyacrylamide gel. Transcripts larger than about 1.5 kb should be run on agarose gels, whereas polyacrylamide gels (4–5%) are better for sizing smaller transcripts. Since secondary structure in the transcript may cause aberrant migration and/or multiple bands, the gel should be run under denaturing conditions. For agarose gels, this means glyoxal or formaldehyde gels, prepared and run according to standard procedures (Molecular Cloning, A Laboratory Manual, 1989). Instructions for preparing and running denaturing acrylamide gels are supplied in section "Recipes" on page 21.

Sample preparation

To get good resolution of the RNA, load ~1 µg per gel lane. For denaturing polyacrylamide gels add an equal volume of Gel Loading Buffer II to each sample, and heat for 3–5 min at 80–90°C. (Gel Loading Buffer II cannot be used with glyoxal agarose gels and it will not completely denature samples run on formaldehyde agarose gels. Use a loading buffer specifically formulated for the type of agarose gel you plan to run.)

To stain the RNA with ethidium bromide during electrophoresis do one of the following:

1. Add 0.5 µg/mL ethidium bromide to the gel mix
2. Add 0.5 µg/mL ethidium bromide to the running buffer
3. Add 10 µg/mL ethidium bromide to the RNA samples (and gel loading buffer) before loading the gel.

(Because single-stranded nucleic acids bind ethidium less efficiently than double-stranded nucleic acids, the fluorescence of RNA samples on a denaturing agarose gel will be less intense than the same amount of DNA.)

Visualizing reaction products

Ethidium bromide stained samples

View ethidium bromide stained gels on a UV transilluminator. Ideally there will be a single, tight band at the expected molecular weight. See section "Multiple reaction products, transcripts of the wrong size" on page 16 for troubleshooting suggestions if this is not what appears on your gel.

Radioactively-labeled transcripts

If the transcription reaction contained a radiolabeled nucleotide tracer (e.g. [α-^{32}P]UTP), the RNA can be visualized by autoradiography. Agarose gels should be dried before exposing to X-ray film, but thin (0.75 mm thick) polyacrylamide gels may be transferred to filter paper, covered with plastic wrap, and exposed directly (when

^{32}P is used). Approximate exposure times for visualizing low specific activity transcripts (e.g. when 1 µL of 800 Ci/mmol, 10 mCi/mL [α-^{32}P] UTP was used in the MEGAscript® reaction) are about 10–30 min with an intensifying screen, or several hours to overnight without a screen, when 1 µL of the undiluted reaction is run on the gel. A recipe for standard denaturing (i.e. 8 M urea) polyacrylamide gels is given in section "Recipes" on page 21.

Optimizing yield of short transcripts

The MEGAscript® Kit is designed to function best with transcription templates larger than ~0.5 kb. Under these conditions, 1 µg of plasmid DNA template per 20 µL reaction gives maximal RNA yield. Increasing the incubation time, template, or polymerase concentration does not generally increase the yield of the reaction. However, with smaller templates, these parameters may require adjustment to maximize reaction yields.

Several types of small transcript templates (<0.5 kb) can be used in MEGAscript® reactions. These include plasmid vectors containing small inserts, PCR products, and synthetic oligonucleotides which can either be entirely double-stranded or mostly single-stranded with a double-stranded promoter sequence (Milligan et al. 1987). Using oligonucleotides, and PCR-derived templates, almost all of the DNA is template sequence, compared to plasmid templates which include non-transcribed vector DNA.

1. Increase the reaction time

   Increasing the incubation time is the easiest variable to change and should be tried first. Try increasing the incubation time to 4 or 6 hr. This allows each RNA polymerase molecule to engage in more initiation events.

2. Increase the template concentration

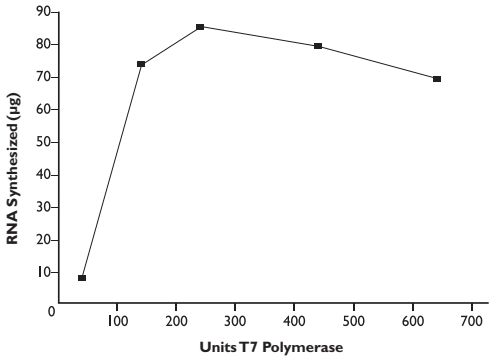
   Increasing the template concentration is the next variable that should be tested. This can be helpful because, with short templates, the initiation step of the transcription reaction is rate limiting. For a 60 nt transcript generated from an 85 bp PCR product, 50 ng of template was found to be saturating. Increasing the amount of template 5 fold, to 250 ng, resulted in only a 30% increase in yield. It is important to remember that 1 µg of a short template contains a much larger molar amount of DNA than 1 µg of a longer template. The 50 ng of template in the above example provided 0.9 pmoles of template (and 0.9 pmoles of promoters), compared to the approximately 0.3 pmoles template in 1 µg of the pTRI-Xef control template. In general, for optimum yield of short transcripts, use about 0.5–2 pmoles of template. For very short templates (i.e. ~20–30 nt), use the upper end of this range.

   If the short template is contained in a plasmid, it may not be possible to add the optimum molar amount. For example, 2 pmoles of template consisting of a 30 bp insert in a 2.8 kb vector would require 4 µg of plasmid DNA. Such large mass amounts of DNA may be detrimental. Thus, it is better to either remove the template from the vector, or to do the transcription reaction under conditions of sub-optimal template concentration.

3. Increase the RNA polymerase concentration

The concentration of RNA polymerase in the kit is optimal for transcription of templates larger than 500 nucleotides, templates coding much smaller transcripts may benefit from adding additional RNA polymerase. Adding 200 units more polymerase may increase yields with very short templates by allowing more initiation events to occur in a given amount of time (see figure 3). We suggest adding high concentration polymerase (e.g. Cat. nos. AM2075, AM2085, and AM2063), ***not*** the 10X Enzyme Mix from the MEGAscript® Kit. Increasing the enzyme should be the last variable tested after increasing incubation time and optimizing template concentration.

Figure 3 RNA Polymerase Titration in MEGAscript® Reaction with an 85 bp Template. MEGAscript® reactions used 200 ng of an 85 bp PCR product that codes a 60 nt RNA. Incubation was for 6 hours, and reaction products were quantitated by measuring the incorporation of a trace amount of ^{32}P-UTP.



Synthesis of capped RNA transcripts

Background

In vivo, most mRNA molecules have a 5' 7-methyl guanosine residue or cap structure which functions in initiation of protein synthesis and protects mRNA from degradation by intracellular nucleases. Capped in vitro transcripts can be synthesized by adding cap analog directly to the MEGAscript® reaction. It is frequently not necessary to cap RNA for in vitro translation experiments. The Retic Lysate IVT™ translation Kit, for example, is supplied with alternative buffers optimized for translating uncapped mRNA.

Note: mMESSAGE mMACHINE® Kits contain cap analog premixed with nucleotides: they are optimized for the synthesis of capped RNAs.

In vitro transcripts which will be microinjected into oocytes or other cells, used for transfection experiments or for in vitro splicing reactions, should generally be capped. The standard strategy to synthesize capped transcripts is to reduce the level of GTP to 10% of the normal concentration and replace the remaining 90% with cap analog (Krieg and Melton, 1987). This results in a high proportion of capped transcripts, but unfortunately it also significantly decreases the yield of the transcription reaction, often to less than 20% of normal yield. To conserve cap analog which is a relatively expensive reagent, and to increase the RNA yield, the ratio of cap analog to GTP can be decreased. Four to one cap:GTP is frequently used, but ratios as low as 1:1 are also used. The table below shows the effect of varying the ratio of cap analog to GTP on the yield of RNA.

Cap analog: GTP ratio	Concentration of cap analog:GTP (mM)	RNA synthesized (µg)	
		3 hour	6 hour
0:1	0: 7.5	91.1	132.6
1:1	3.75: 3.75	87.6	132.6
2:1	5: 2.5	58.2	60.3
4:1	6: 1.5	43.1	43.8
8:1	6.67: 0.83	27.2	28.2
10:1	6.82: 0.68	24.6	25.5

In this experiment, the quantity of RNA synthesized depended on the concentration of GTP in the reaction. When the concentration of GTP is reduced from 7.5 mM to 3.75 mM the yield of RNA was unaffected, but reducing the GTP to 2.5 mM reduced RNA yield by approximately 50%. Further decreases in GTP concentration resulted in even larger decreases in yield. A similar experiment is shown in Figure 5 except that a template coding for the smaller ß-globin mRNA was used.

Figure 4 Effect of Cap Analog:GTP Ratios on the Yield of Globin RNA. Globin mRNA was synthesized in T7-MEGAscript® reactions in which the concentration of cap analog was varied in a similar manner as shown in the table on the previous page. The reactions were incubated at 37°C using 1 µg of T7-globin template DNA (0.5 kb insert).

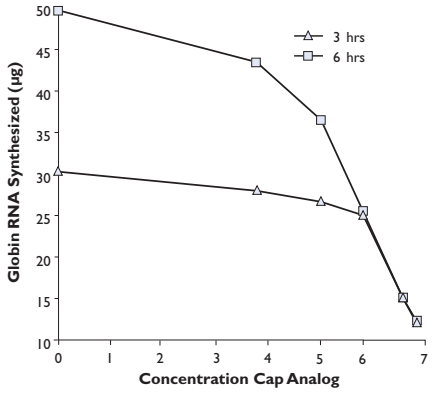
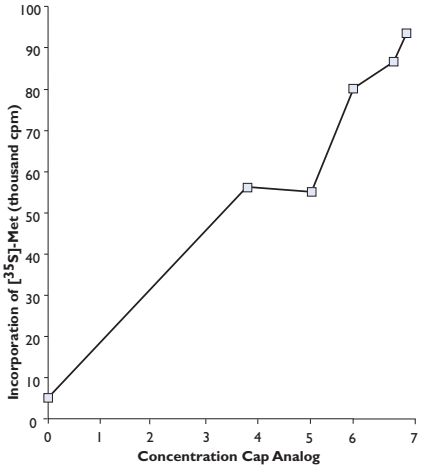


Figure 5 Translation of Globin RNAs from Figure 4. Globin mRNA (6 µg/mL, a sub-saturating level) was translated in with Retic Lysate IVT™ for 60 minutes at 30°C with 12.5 µCi of [^{35}S]-methionine (1200 Ci/mmol). The incorporation of acid-precipitable cpm shown is the average of two duplicate samples.



With both templates, RNA yield is dramatically reduced at levels of cap analog at 6 mM and GTP at 1.5 mM (a 4:1 ratio). These are the conditions we recommend for most capped RNA synthesis reactions because at least 80% of the RNA synthesized will be capped. Although the ratio of capped RNAs synthesized can be increased by increasing the ratio of cap analog to GTP, the reduced yields don't generally justify it. Two additional points worth noting. The total yield of RNA was higher with the larger template. Also the transcription reaction goes to completion sooner with the larger template. Therefore, it may be desirable to adjust the reaction time depending on the length of the transcript and the concentration of GTP in the reaction. The translation of globin mRNA in a reticulocyte lysate is known to be very dependent on the presence of a 5' cap. Notice that in the experiment shown in figure 5, at a 4:1 ratio of cap analog to GTP the translational efficiency was only 15% lower than with a 10:1 ratio.

Reaction set-up

The reaction setup that follows uses a 4:1 ratio of cap analog to GTP.

1. Assemble the reaction at room temperature in the order shown: the final volume is 20 µL.

   IMPORTANT! Spermidine in the Reaction Buffer can lead to precipitation of the template DNA if the reaction is assembled on ice.

   Note: For convenience, equal volumes of the four ribonucleotide solutions can be mixed together and 8 µL added to a standard 20 µL reaction as one component instead of adding 2 µL of each of the four separate ribonucleotide solutions.

Volume	Component
to 20 µL	Nuclease-free water
2 µL	ATP solution (75 mM T7 or T3; 50 mM SP6)
2 µL	CTP solution (75 mM T7 or T3; 50 mM SP6)
2 µL	UTP solution (75 mM T7 or T3; 50 mM SP6)
2 µL	1:5 dilution of GTP solution (15 mM T7 or T3; 10 mM SP6)
to 4–6 mM	Cap Analog (6 mM for T7 or T3; 4 mM for SP6): 3 µL of 40 mM stock = 6 mM 2 µL of 40 mM stock = 4 mM
2 µL	10X Reaction Buffer
(0.25–0.5 µL)	(optional) labeled rNTP as a tracer, e.g. [α-^{32}P]UTP (any specific activity is acceptable)
-- µL	1 µg linearized template DNA
2 µL	Enzyme Mix

2. Mix contents by flicking the tube or mixing with a pipettor and then microfuge tube briefly to collect the reaction mixture at the bottom of the tube.
3. Incubate 2–4 hours and continue the procedure from step 4. on page 10.

Using the MEGAscript® Kit to make RNA probes

The MEGAscript® Kit can be used to transcribe low specific activity radiolabeled probes, and non-isotopically labeled RNA probes. The MEGAscript® Kit should not be used to prepare RNA probes that are radiolabeled to high specific activity, because the kit is not configured to transcribe RNA efficiently in conditions of very low nucleotide concentrations – use the MAXIscript® Kit instead.

Synthesis of low specific activity radiolabeled probes

RNA probes that are radiolabeled to low specific activity are used to detect very abundant RNA species such as ribosomal RNAs, or overexpressed messages. We do not include a procedure here because the amount of radiolabel required in the reaction varies greatly depending on the specific activity needed. In general though, the ribonucleotide concentration used with MEGAscript® Kits should not be changed, in other words, use 7.5 mM of each NTP for T7 and T3 Kits, and 5 mM for SP6 Kits. Any radiolabel added should contribute to this amount.

Synthesis of nonisotopically labeled probes

The MEGAscript® Kit can be used to synthesize large amounts of both biotinylated and digoxigenin-labeled probes. These reactions are set up with a mixture of labeled and unlabeled nucleotides at typical MEGAscript® nucleotide concentrations. Biotin- or digoxigenin-modified nucleotides should be used at a ratio of 1:2 or 1:3 with standard nucleotide. For most efficient incorporation, the pH of nucleotide solutions should be close to neutrality. Gel purification or precipitation with LiCl or NH4OAc and ethanol can be used to remove unincorporated nucleotides.

Spin column preparation and use

Unincorporated labeled nucleotides can be removed by size exclusion chromatography on RNase-free Sephadex G-25 or G-50 spin columns. The following is a procedure for the preparation and use of spin columns:

1. Resuspend and equilibrate Sephadex G-25 or G-50 with 2 volumes of TE (10 mM Tris-HCL, pH 8.0, 1 mM EDTA), then wash with several volumes of TE.
2. Place the resuspended and washed resin in 1.5 volumes of TE in a glass bottle and autoclave. Store at 4°C until use.
3. Rinse a 1–3 mL spin column thoroughly with distilled water; frits may be pre-installed, or made by plugging the bottom of a 1 mL syringe with a support such as siliconized glass beads.
4. Pipet 1–3 mL of the prepared, well mixed resin into the washed spin column. Place the column in a 15 mL plastic centrifuge tube and spin at 2,000 rpm for 10 min in a centrifuge with a swinging-bucket rotor.
5. Place the end of the spin column containing the spun resin into an appropriate microfuge tube (typically, 0.5 mL) and insert the assembly into a new 15 mL centrifuge tube.
6. Load 20–100 µL of the sample onto the center of the resin bed (dilute sample with nuclease-free water or TE Buffer if necessary), and spin at 2,000 rpm for 10 min. The eluate collected in the microfuge tube should be approximately the same volume as the sample loaded onto the column, and it will contain about 75% of the nucleic acid applied to the column.

IMPORTANT! It is important that the centrifugation conditions for column packing and sample purification be identical; varying them could lead to either incomplete recovery or dilution of the sample. The spin column can be tested by loading 100 µL of TE onto it and centrifuging: 100 µL of eluate should be recovered. If recovery is much greater or less than 100 µL, the column is not equilibrated and should be tested again.

D

Safety

General safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:
Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.).
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of

according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:
- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:
- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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Documentation and Support

Obtaining SDSs

Safety Data d PRoduSheets (SDSs) are available from **www.lifetechnologies.com/support.**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support.**

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