UniScript™ T3 Transcription Kit, Product No. **US-T3**
UniScript™ T7 Transcription Kit, Product No. **US-T7**
UniScript™ SP6 Transcription Kit, Product No. **US-SP6**
UniScript™ T7/SP6 Transcription Kit, Product No. **US-SP6-T7**
UniScript™ T7/T3 Transcription Kit, Product No. **US-T3-T7**
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ProductInformation

TECHNICAL BULLETIN

Product Description

UniScript™ Transcription Kits are the most convenient, versatile and efficient method for producing radioactively and non-radioactively labeled probes and unlabeled RNA by *in vitro* transcription. These kits utilize SP6, T3 and/or T7 phage RNA polymerases, which are widely utilized for *in vitro* transcription due to a high specificity for their respective promoters. Protocols are provided for producing non-radioactively labeled RNA transcripts utilizing biotin-11-CTP, biotin-16-UTP, fluorescein-12-UTP and digoxigenin-11-UTP (labeled nucleotides not provided in kit). The transcripts can be used in many applications, including blot hybridizations, RNase protection assays and *in vitro* translation.

Reagents Provided

Sufficient for 60 reactions of labeled (CTP or UTP) or unlabeled transcripts

- 5X Minus CTP Reaction Mix, Product No. C2099 — 0.25 ml
  200 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10 mM spermidine, 30 mM $MgCl_2$, 50 mM DTT, 2.5 mM ATP, 2.5 mM GTP, 2.5 mM UTP
- 10 mM CTP, Product No. C2688 — 1 vial (65 µl)
- 50 µM CTP, Product No. C2224 — 0.3 ml
- 5X Minus UTP Reaction Mix, Product No. U2127 — 0.25 ml
  200 mM Tris-HCl, pH 7.6, 50 mM NaCl, 10 mM spermidine, 30 mM $MgCl_2$, 50 mM DTT, 2.5 mM ATP, 2.5 mM CTP, 2.5 mM GTP
- 10 mM UTP, Product No. U7255 — 1 vial (65 µl)
- 50 µM UTP, Product No. U5002 — 0.3 ml
- DNase I, RNase-free, 1 units/µl, Product No. D5307 — 1 vial (65 µl)
- RNA Gel Loading Buffer, Product No. R4268 — 1 ml
  62.5% (v/v) deionized formamide, 1.14 M formaldehyde, 200 µg/ml bromophenol blue, 200 µg/ml xylene cyanole, 1.25X MESA buffer, 50 µg/ml ethidium bromide
- pTRI-GAPDH-Mouse Antisense Control Template, 0.25 µg/µl, Product No. C1974 — 0.01 ml
- Molecular Biology Grade Water, Product No. W4502 — 1 ml

For UniScript T3 Transcription Kit Only

- T3 RNA Polymerase/RNase Inhibitor Mix, Product No. R0774 — 2 x 1 vial (65 µl)
  15 units/µl T3 RNA polymerase, 5 units/µl RNase inhibitor

For UniScript T7 Transcription Kit Only

- T7 RNA Polymerase/RNase Inhibitor Mix, Product No. R0399 — 2 x 1 vial (65 µl)
  15 units/µl T7 RNA polymerase, 5 units/µl RNase inhibitor

For UniScript SP6 Transcription Kit Only

- SP6 RNA Polymerase/RNase Inhibitor Mix, Product No. R0649 — 2 x 1 vial (65 µl)
  15 units/µl SP6 RNA polymerase, 5 units/µl RNase inhibitor

For UniScript T7/SP6 Transcription Kit Only

- T7 RNA Polymerase/RNase Inhibitor Mix, Product No. R0399 — 1 x 1 vial (65 µl)
- SP6 RNA Polymerase/RNase Inhibitor Mix, Product No. R0649 — 1 x 1 vial (65 µl)

For UniScript T7/T3 Transcription Kit Only

- T7 RNA Polymerase/RNase Inhibitor Mix, Product No. R0399 — 1 x 1 vial (65 µl)
- T3 RNA Polymerase/RNase Inhibitor Mix, Product No. R0774 — 1 x 1 vial (65 µl)

Precautions and Disclaimer
Sigma's UniScript Transcription Kits are for laboratory use only. Not for drug, household or other uses.

Storage/Stability
Store all reagents at –20ºC. Under proper storage conditions, kit reagents are stable for 1 year from date of receipt.

Procedure
The UniScript Transcription Kits can be used for making unlabeled transcripts or transcripts labeled with radioactively or non-radioactively labeled CTP or UTP. Procedures for each type of reaction are outlined below.

A. Template DNA Preparation
Linearized template DNA is required for run-off transcription. These templates can be plasmids linearized by restriction digest or the use of purified PCR products. The templates should be sufficiently concentrated to allow the addition of 1 µg of DNA to the transcription reaction in ≤5 µl. In addition, template DNA needs to be free from contaminating nucleases. This can be accomplished using the GenElute™ PCR DNA Purification Kit (Product No. GEN-PCR).

B. Transcription Reactions
Directions for making unlabeled, radioactively labeled and non-radioactively labeled probes are outlined below.

- Unlabeled transcripts can be used in various applications, including *in vitro* translation. Reactions for unlabeled transcripts can be prepared using either the 5X Minus CTP Reaction Mix in combination with 10 mM CTP or with the 5X Minus UTP Reaction Mix in conjunction with 10 mM UTP.

- Non-radioactively labeled transcripts can be synthesized in a similar manner utilizing a mixture of labeled and unlabeled CTP or UTP in conjunction with the appropriate 5X Reaction Mix.

- Radioactively labeled transcripts can be synthesized to high specific activity and high efficiency using the appropriate 5X Reaction Mix and the procedure outlined below. For full-length transcripts (e.g. for RNase protection assays), unlabeled (cold) nucleotide may need to be added as suggested in the procedure. The requirement of cold nucleotide for full-length transcripts must be determined empirically for each template.

1. Remove reagents required for each reaction. Place enzyme solutions on ice immediately. The other tubes should be thawed at 37ºC for ~5 minutes, mixed and then stored on ice (except the water, which should be stored at room temperature).
2. Add the reagents for the appropriate reaction, <u>in order listed in the tables below</u>, to an appropriately labeled microcentrifuge tube at room temperature. Addition of reagents in a different order may cause precipitation of the template DNA and reduce the efficiency of transcription. After all of the reagents have been added, pipette the entire contents of the tube up and down to mix.

<u>Radioactively Labeled Transcription Reactions</u>
For applications requiring full-length transcripts (e.g. for RNase protection assay), cold nucleotide may need to be added to a final concentration of 2-10 µM. It is important to note that the addition of cold nucleotide will reduce the sensitivity of the labeled probe. Therefore, the smallest amount of cold nucleotide should be added to allow transcription of full-length products. Probes that will be used in hybridizations to blots do not need to be full-length and should be synthesized without the addition of cold nucleotide to maximize specific activity. Any α-labeled (including ^{32}P, ^{33}P and ^{35}S) or ^{3}H-labeled ribonucleotide triphosphate (UTP or CTP) can be used for labeling. Reactions should be set up as shown in Table 1.

Table 1. Preparation of Radioactively Labeled Probes

Reagent	Volume	Final Concentration
RNase-free water	to 20 µl	----
Linearized template DNA	1 µg	50 ng/µl
5X Reaction mix (minus UTP or CTP)	4 µl	1X
[α-^{32}P] UTP or CTP[(a)]	5 µl	3 µM
RNA polymerase/RNase inhibitor	2 µl	1.5/0.5 units per µl
Total volume	20 µl	

a) The use of labeled nucleotide at 800 Ci/mmole and 10 mCi/ml will yield full-length probes for most templates. The use of nucleotides at higher specific activity may require the addition of cold nucleotide to bring the final limiting nucleotide concentration to >3 µM.

Non-Radioactively Labeled Transcription Reactions

This procedure can be used for labeling transcripts with any non-radioactively labeled ribonucleotide triphosphate (UTP or CTP). These kits have been optimized for the incorporation of the following labeled nucleotides: Biotin-11-CTP, Biotin-11-UTP, Biotin-16-UTP, Fluorescein-12-UTP and Digoxigenin-11-UTP. Other labeled nucleotides can also be used with these kits although the optimal ratio of labeled and unlabeled nucleotide must be determined empirically. Listed in Table 2 are nucleotide ratios that function well for these labeled nucleotides in combination with a wide variety of transcription templates (in all cases, the total volume of unlabeled and labeled nucleotide should be 1 µl per 20 µl reaction mixture).

Table 2. Recommended Ratios of Labeled to Unlabeled Nucleotide

Labeled Nucleotide	Labeled:Unlabeled Ratio
Biotin-11-CTP (Sigma Product No. B7048)	50:50
Biotin-11-UTP (Sigma Product No. B8280)	50:50
Biotin-16-UTP (Sigma Product No. B6923)	50:50
Fluorescein-12-UTP (Sigma Product No. F5921)	50:50
Digoxigenin-11-UTP (Roche Product No. 1209256)	35:65

In cases where the nucleotide mix will be used repeatedly, it is convenient to prepare a nucleotide premix (e.g. mix 10 µl 10 mM Biotin-16-UTP with 10 µl 10 mM UTP to prepare a premix suitable for 20 transcription reactions). Reactions should be set up as shown in Table 3.

Table 3. Preparation of Non-radioactively Labeled Probes

Reagent	Volume	Final Concentration
RNase-free water	to 20 µl	----
Linearized template DNA	1 µg	50 ng/µl
5X Reaction mix (minus UTP or CTP)	4 µl	1X
10 mM total UTP or CTP (mix of labeled and unlabeled ribonucleotides)	1 µl	500 µM total
RNA polymerase/RNase inhibitor	2 µl	1.5/0.5 units per µl
Total volume	20 µl	

Unlabeled Transcription Reactions

Reactions can be scaled up to increase yields. Up to 10 µg RNA (~500 bases in length) can be obtained from a 20 µl reaction. The yield is increase to ~40 µg by scaling the reaction to 100 µl. Reactions should be set up as shown in Table 4.

Table 3. Preparation of Unlabeled Probes

Reagent	Volume	Final Concentration
RNase-free water	to 20 µl	----
Linearized template DNA	1 µg	50 ng/µl
5X Reaction mix (minus UTP or CTP)	4 µl	1X
10 mM CTP or UTP	1 µl	500 µM
RNA polymerase/RNase inhibitor	2 µl	1.5/0.5 units per µl
Total volume	20 µl	

3. Incubate reactions at least 1.5 hours at 37ºC.
4. Add 1 µl RNase-free DNase I and incubate 15 minutes longer at 37ºC. This step degrades the template DNA.
5. At this point the RNA can be purified away from unincorporated nucleotides, buffer and enzymes by several methods:
   a. Using the GenElute™ Mammalian Total RNA Kit (Product Nos. RTN-10, RTN-70 or RTN-350).
   b. By filtration on SigmaSpin™ Post Reaction Purification Columns (Product No. S5059).
   c. By phenol/chloroform extraction and/or ethanol precipitation as described by Sambrook *et al*.[1]

d. If full-length transcripts are required, the transcripts should be gel purified as described in Section C, Gel Purification of Probes.

Non-radioactively labeled probes can be utilized in probing experiments without prior purification. Sigma does not recommend purifying biotin-labeled transcripts by method (a), as we have observed high backgrounds in downstream blotting applications when biotinylated probes are purified in this manner. This background has not been observed with fluorescein- or digoxigenin-labeled probes.

Control Transcription Reactions

Control reactions can be prepared for any of the labeling methods described in the previous section, utilizing 2-4 µl (0.5-1 µg) of the provided pTRI-GAPDH control template per 20 µl reaction mixture. This linearized template contains a 316 bp fragment of the mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene derived from exons 5-8 (nucleotides 345-660 of Accession No. M32599) inserted downstream of tandem SP6, T7 and T3 promoters. When transcribed with the following RNA polymerases, antisense transcripts of the following lengths are produced:

SP6	→	413 bases
T7	→	383 bases
T3	→	355 bases

C. Gel Purification of Probes

For applications such as RNase protection and S1 nuclease assays, full-length transcript should be isolated by gel purification. The following procedure describes the process of gel purifying radiolabeled transcripts.[2] This same procedure may be used to purify unlabeled and non-radioactively labeled probes visualized by UV shadowing.[1]

1. After treatment of reactions with DNase I, add an equal volume (20 µl) of RNA gel loading buffer.
2. Heat the mixture at 70ºC for 10 minutes.
3. Load the entire sample in a single well on a 5% polyacrylamide, 8 M urea, 1X TBE gel (~1 mm thick) and electrophorese in 1X TBE at 200 V just until the bromophenol blue runs off the bottom of the gel.
4. Remove one glass plate from the gel, cover with plastic wrap and expose to film for 1-10 minutes. It is important to mark the gel for orientation so that the developed film can be aligned with the gel accurately.
5. Develop the film as per manufacturer's instructions and align the film to the gel. Place the film upside down on a light box or white piece of paper. Invert the gel and align properly on top of film. Looking through the glass plate and gel, use a marker to circle the band corresponding to the slowest migrating, most intense band on the film.
6. Cut bands from gel by turning the gel over and remove the film. Cut out the areas of the gel outlined by marker and place each gel fragment in a separate tube containing 0.5 M ammonium acetate, 1 mM EDTA and 0.1% SDS.
7. Allow the transcripts to elute from the gel for 3 hours at room temperature or overnight at 4ºC.
8. Transfer the eluate to a fresh tube and measure a small amount by scintillation counting.

References

1. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, second edition, Cold Spring Harbor Laboratory Press, New York
2. Krieg, P.A. (1991) *Methods in Gene Technology*. Volume 1: pages 35-62.

Related Products

Product Name	Product Number
Biobond™ Nylon Membrane	N1406, N4031, N3656, N3781, N3906
Biobond-Plus™ Nylon Membrane	N5281, N5781, N5406, N5531, N5656
Precast Agarose Gels, 1.25% in MOPS Buffer for RNA analysis	P6222
PerfectHyb Plus™ Hybridization Buffer	H7033
CDP-*Star*™ Universal Detection kit	U-ALK
CDP-*Star*™ Ready-to-Use	C0712