2nd Edition

RNA Guide

Carrying the Message

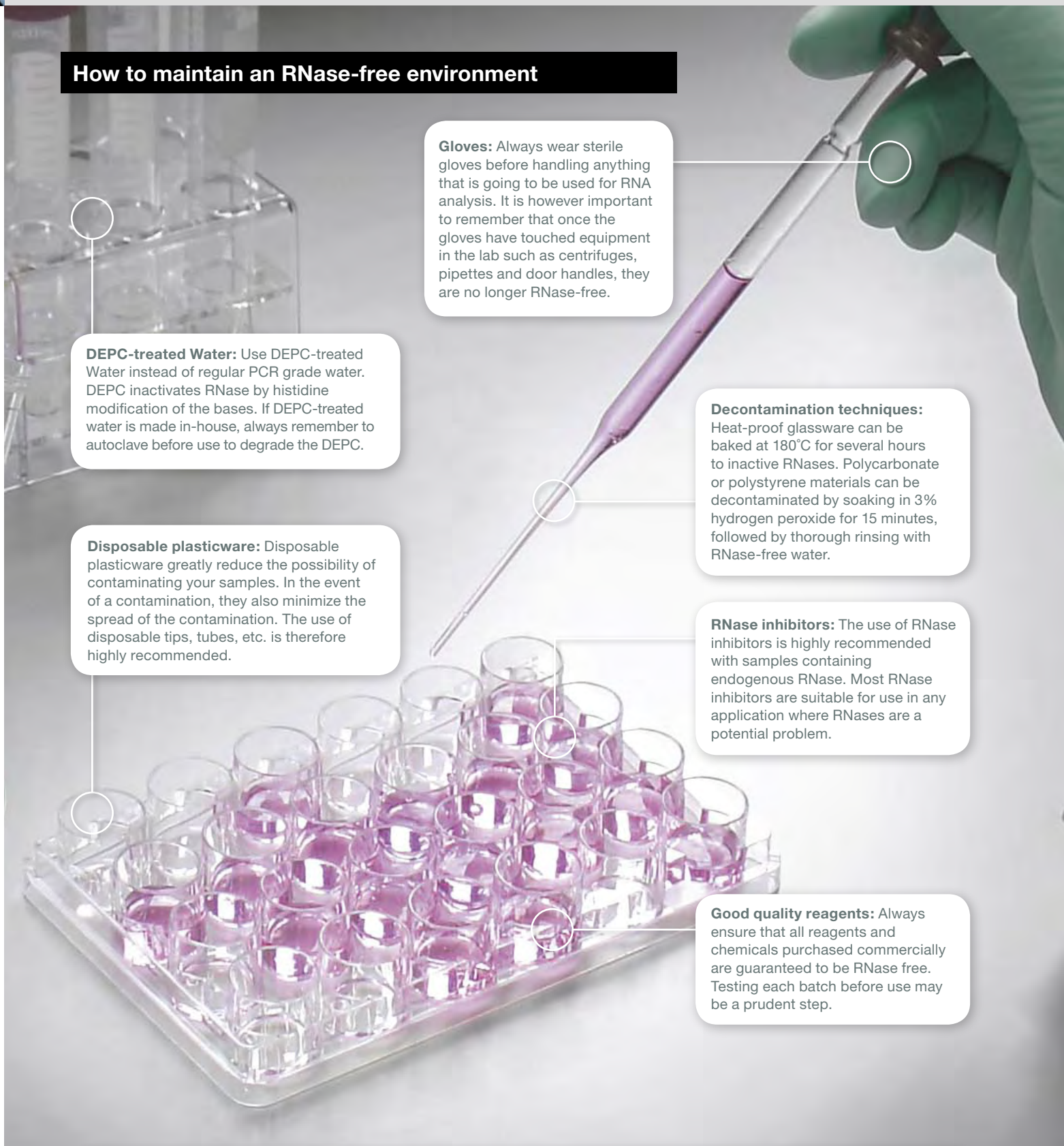
BIOLINE

An RNase free environment is essential when working with RNA samples.

In the laboratory, obtaining full length, high quality RNA often proves to be a daunting task. There are two main reasons for RNA degradation during RNA analysis. Firstly, RNA, by its very structure, is inherently weaker than DNA. RNA is made up of ribose units, which have a highly reactive hydroxyl group on C2 that takes part in RNA-mediated enzymatic events. This makes RNA more chemically labile than DNA. RNA is also more prone to heat degradation than DNA. Secondly, enzymes that degrade RNA, ribonucleases (RNases) are so ubiquitous and hardy, that eliminating them often proves to be virtually impossible. For example, autoclaving a solution containing bacteria will destroy the bacterial cells, but not the RNases released from the cells.

Contents

How to maintain an RNase-free environment



Gloves: Always wear sterile gloves before handling anything that is going to be used for RNA analysis. It is however important to remember that once the gloves have touched equipment in the lab such as centrifuges, pipettes and door handles, they are no longer RNase-free.

DEPC-treated Water: Use DEPC-treated Water instead of regular PCR grade water. DEPC inactivates RNase by histidine modification of the bases. If DEPC-treated water is made in-house, always remember to autoclave before use to degrade the DEPC.

Decontamination techniques: Heat-proof glassware can be baked at 180˚C for several hours to inactive RNases. Polycarbonate or polystyrene materials can be decontaminated by soaking in 3% hydrogen peroxide for 15 minutes, followed by thorough rinsing with RNase-free water.

Disposable plasticware: Disposable plasticware greatly reduce the possibility of contaminating your samples. In the event of a contamination, they also minimize the spread of the contamination. The use of disposable tips, tubes, etc. is therefore highly recommended.

RNase inhibitors: The use of RNase inhibitors is highly recommended with samples containing endogenous RNase. Most RNase inhibitors are suitable for use in any application where RNases are a potential problem.

Good quality reagents: Always ensure that all reagents and chemicals purchased commercially are guaranteed to be RNase free. Testing each batch before use may be a prudent step.

Correct storage of RNA is also very important to avoid RNA degradation. In the short term, RNA may be stored in RNase-free H_2O or TE buffer at -80˚C for 1 year without degradation. For long term storage RNA samples may be stored as ethanol precipitates at -20˚C. However, when dissolved in ethanol, RNA is not dispersed evenly in the solution and cannot be used directly in quantitative experiments. Instead, precipitates should be pelleted and redissolved in an aqueous buffer before pipetting.

Sources of RNase

Skin: The presence of RNases on human skin surfaces has been well documented. RNase contamination through this source is very easy to acquire and spread if tubes, pipette tips, bench tops, etc. are touched with bare hands.

Dust: Dust particles floating in the air often harbor bacteria or mold. The RNases from these microorganisms get deposited wherever the dust settles. This includes lab equipment, open bottles, etc.

Reagents: If the reagents used for RNA analysis are not certified to be RNase-free, there is a good chance that some of the contamination will come from this source. Reagents can also become contaminated in the lab itself if proper care is not taken.

Samples: RNase contamination can come from the samples themselves as tissues and cells contain endogenous RNases.

Determination of RNA yield, purity and integrity

The yield of total RNA may be determined spectrophotometrically at 260nm, whereby 1 unit of absorbance (A_{260}) = 40µg of single stranded RNA/ml. The purity can also be determined spectrophotometrically from the ratio of the relative absorbances at 260 and 280nm. Good quality RNA will have a A_{260}/A_{280} ratio in the range of 1.7 to 2.1.

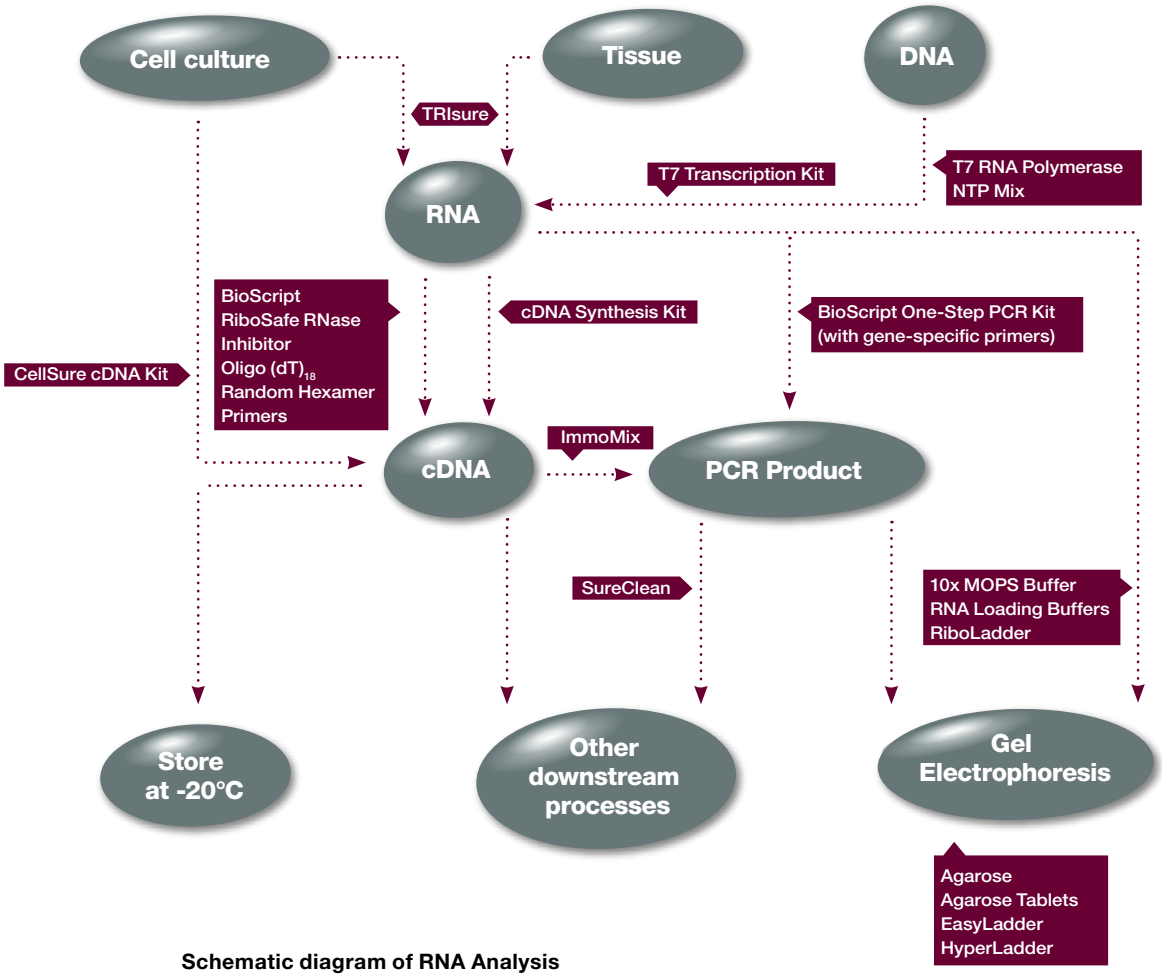
The most common procedure for determining RNA integrity is running 2-4µg of a total RNA sample on an agarose denaturing gel. The RNA may be visualized by EtBr staining, which reveals the ribosomal RNA bands. These bands can vary depending on the organism the RNA was extracted from (see table). In general, for good quality RNA the bands should be distinct, with no smearing underneath them and the 28S band should be approximately twice as intense as the 18S band.

Ribosomal RNA Sizes

Species	rRNA	Size(Kb)
Human	18S	1.9
	28S	5.0
Mouse	18S	1.9
	28S	4.0
Drosophila	18S	2.0
	28S	4.1
Tobacco Leaf	16S	1.5
	18S	1.9
	23S	2.9
	25S	3.7
Yeast	18S	2.0
(*S. cerevisiae*)	26S	3.8
E. coli	16S	1.5
	23S	2.9
Xenopus	18S	1.8
	28S	4.0
Worm	18S	1.7
(*C. elegans*)	28S	3.5

RNA Analysis

RNA analysis frequently involves the detection of RNA transcripts. In order to detect RNA transcripts, the RNA is initially amplified by reverse transcription to produce complementary DNA (cDNA). The cDNA is then used to carry out numerous applications including traditional PCR amplification, real-time PCR and DNA microarray experiments. The schematic diagram below shows the various steps involved in RNA analysis. The corresponding Bioline products are indicated in purple. Bioline's range of RNA Analysis products are manufactured and packaged under the most stringent conditions and are guaranteed to be RNase/DNase free.



Schematic diagram of RNA Analysis

RNA Isolation

The first step to successful RNA analysis is the isolation of pure, intact high-quality RNA. The quality of the isolated RNA can have a tremendous effect on downstream processes such as quantitative PCR and microarray analysis. For isolation of high quality RNA from animal tissue, plant tissue or cultured cells, we recommend TRIsure™.

TRIsure™

A ready-to-use reagent for the isolation of total RNA from cells and tissue

FEATURES

- Quick isolation of high-quality RNA
- Ready-to-use solution for a wide variety cells and tissues
- Cost-effective
- Convenient 1hour protocol
- Performs well with large or small amounts of tissue or cells

APPLICATIONS

- Isolation of high quality RNA from diverse biological material, including animal and plant tissues rich in polysaccharides and proteoglycans
- Purified RNA is ideal for any downstream application such as RT-PCR or *in vitro* translation

PACK SIZE	CAT NO.
100ml	BIO-38032
200ml	BIO-38033

TRIsure™ is a ready-to-use reagent for the isolation of total RNA from cells and tissue. TRIsure maintains the integrity of the extracted RNA, while disrupting cells and subsequently dissolving cell components. The biological sample is homogenized or lysed in TRIsure and then separated into organic and aqueous phases. The RNA remains in the aqueous phase and is subsequently recovered by precipitation with isopropyl alcohol.

The isolated RNA is suitable for any downstream application such as RT-PCR, hybridization assays, or *in vitro* translation. 1ml of TRIsure is sufficient to isolate Total RNA from 1 x 10^7 cells or 100mg of tissue.

From 1mg of tissue or 1 x 10^6 of cultured cells, the expected yield of RNA is:

- 2-5µg from mouse kidney (tissue)
- 5-10µg from mouse liver (tissue)
- 8-15µg from epithelial cells (cultured cells)
- 20-25µg from fibroblasts (cultured cells)

PROTOCOL

Reagents Required (not supplied):

- Chloroform
- Isopropyl alcohol
- 75% Ethanol (in DEPC-treated Water)
- DEPC-treated Water

1. Homogenization.

Tissue: Homogenize tissue samples in 1ml of TRIsure per 50-100mg of tissue. For small quantities of tissue (1-10mg), add 800µl of TRIsure. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed.

Plant Tissue: Following homogenization, insoluble material is removed by centrifugation at 12,000 x g for 10 minutes at 2-8˚C. Transfer the cleared homogenate to a fresh tube.

Cells Grown on Monolayer: Lyse cells directly in a culture dish or flask by adding 1ml of TRIsure per 10cm^2 growth area, pipette the cell lysate several times to ensure sufficient cell disruption.

Cells Grown in Suspension: Pellet cells at 200 x g for 5 minutes at room temperature. Lyse cells with 1ml of TRIsure per 5 x 10^6 cells and pass the lysate several times through a pipette tip. For small quantities of cells (10^2 -10^6), lyse cells in 800µl of TRIsure.

Note: At this stage, samples can be stored for at least one month at -60 to -70˚C.

2. Phase Separation.
Incubate samples for 5 minutes at room temperature. Add 0.2ml of chloroform per 1ml of TRIsure used. Cap tubes securely and shake vigorously by hand for 15 seconds.
Incubate samples for 2-3 minutes at room temperature. Centrifuge samples at 12,000 x g for 15 minutes (or 2600 x g for 20-30 minutes) at 2-8˚C. The sample will separate into a pale green, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase that contains the RNA.

3. RNA Precipitation.
Transfer the aqueous phase to another tube. Precipitate the RNA by mixing with isopropyl alcohol. Use 0.5ml of isopropyl alcohol per 1ml of TRIsure used. Incubate samples for 10 minutes at room temperature then centrifuge at 12,000 x g for 10 minutes (or 2600 x g for 20-30 minutes) at 2-8˚C.
Note: For small quantities of tissue/cells, RNase-free Glycogen Co-precipitant (BIO-37077) can be added to the aqueous phase before addition of isopropyl alcohol to aid RNA precipitation. Add 5-10µg of Glycogen per 800µl of TRIsure.

4. RNA Wash.
Remove the supernatant. Wash the pellet once with 75% ethanol, adding at least 1ml of ethanol per 1ml of TRIsure used. Vortex samples and centrifuge at 7500 x g for 5 minutes at 2-8˚C.
Note: At this stage, samples can be stored for one week at 2-8˚C, or 12 months at -5 to -20˚C.

5. Re-dissolving the RNA.
Air-dry the pellet for 5-10 minutes. Dissolve in DEPC-treated Water (BIO-38030) by pipetting the solution up and down, and incubating for 10 minutes at 55-60˚C. Store RNA at -70˚C.

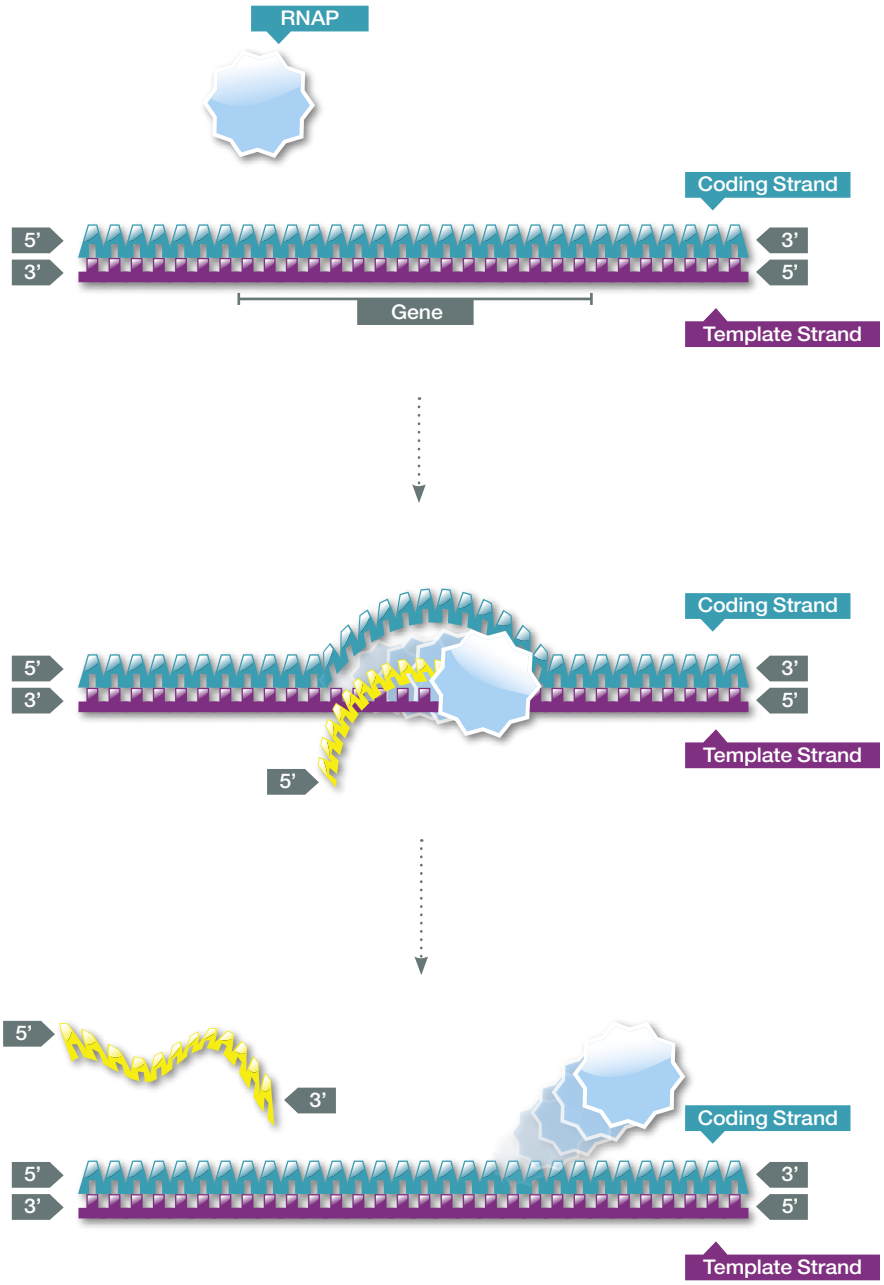
RNA ISOLATION TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION(S)
Genomic DNA contamination *	Insufficient volume of TRIsure used	Ensure that 1ml TRIsure™ per 10cm^2 area of cells or 5 x 10^6 cells is used. If problem persists, increase TRIsure volume by 1.5x
	Incomplete lysis or homogenization	Homogenize tissue thoroughly and centrifuge to remove insoluble material. Pipette resultant cell lysate up and down thoroughly until it becomes visually less viscous
	Contamination of interphase layer during separation of the RNA-containing aqueous layer	Pipette off the aqueous phase very carefully. It is important that none of the white interphase is transferred into your RNA sample, so we recommend that you leave the lower part of the aqueous phase intact
Low RNA yield	Loss of pellet	If starting sample is small, the RNA pellet may not be easily visualized after isopropyl alcohol precipitation, so care must be taken when removing the supernatant from the pellet
	Incomplete lysis or homogenization	Homogenize tissue thoroughly and centrifuge to remove insoluble material. Pipette resultant cell lysate up and down thoroughly until it becomes visually less viscous
	Incomplete solubilization of final RNA pellet	Ensure RNA pellet is completely dissolved in solution
RNA degradation	RNase contamination	The protocol must be carried out carefully in a DNA-free, RNase-free environment. Ensure all pipettes, tips, tubes and work areas are free from RNases and wear gloves. Addition of RNase Inhibitor (BIO-65028) to the extracted RNA sample can help prevent degradation of the sample

** If downstream applications could be affected by small amounts of DNA, we recommend an additional step of treating the RNA sample with DNase I.*

RNA Synthesis

RNA synthesis (or transcription) is carried out by RNA polymerase. The polymerase requires, in addition to a DNA template, all four ribonucleoside 5'-triphosphates and Mg^{2+}. Only one strand of the double-stranded DNA, known as the non-coding or template strand, serves as the template. The process of transcription can be divided into three main steps: initiation, elongation and termination.



Schematic representation of RNA Synthesis

Initiation:
Initiation begins when the RNA polymerase binds to a promoter DNA sequence and begins to unwind small sections of the strands (12-17 bases at a time), forming a transcription bubble. Various initiation factors are also involved in the process.

Elongation:
The transcription bubble moves along the DNA strand, reading the template strand in the 3'-5' direction and incorporating NTPs into the growing RNA strand. The DNA is rewound as the bubble proceeds, the RNA-DNA hybrid is displaced and the RNA strand is extruded.

Termination:
Specific sequences in the DNA signal the termination of RNA synthesis. When the polymerase encounters such a sequence, it pauses and facilitates the dissociation of the transcript.

T7 RNA Polymerase

A DNA-dependent RNA polymerase

FEATURES

- High stringency for the T7 promoter
- High level of purity, specificity and activity
- Readily incorporates NTPs
- Available as T7 Transcription Kit

APPLICATIONS

- Production of RNA and double-stranded RNA for siRNA experiments
- Generation of radioactive RNA probes with high specific activity
- Synthesis of RNA for RNA-protein interaction, splicing and processing studies
- RNA production for *in vitro* translation

Reaction Conditions (for a 20µl volume)

5x Ultimate Reaction Buffer (supplied)	4µl
DNA Template (0.3µg/µl)	1.0µl
DTT 1M *	0.8µl
T7 RNA Polymerase	0.7µl
Optional RNase Inhibitor *	
NTP Mix, 100mM *	0.8µl
DEPC-treated Water *	up to 20µl

* These reagents are not supplied and can be purchased separately from Bioline. A complete kit containing all reagents is also available (BIO-21072).

Reactions should be incubated between 37-42˚C for 2-4 hours dependent upon fragment size.
This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

CONC.	PACK SIZE	CAT NO.
20u/µl	5000 Units	BIO-21055

T7 RNA Polymerase is a DNA-dependent RNA polymerase, which catalyzes RNA synthesis in the 5'-3' direction downstream from any DNA with a T7 promoter sequence. T7 RNA Polymerase can be used to produce RNA from cloned inserts and will incorporate labeled NTPs. Products of this enzyme can be used as probes for screening purposes.

Includes the Bioline 2x Ultimate Reaction Buffer, a proprietary buffer specially formulated and optimized to give exceptional performance and reliability.

T7 Promoter Sequence
The binding and transcription initiation of T7 RNA Polymerase is dependent upon the presence of a T7 promoter sequence:

5' TAATACGACTCACTATAGGGAGA 3'

The red sequence represents the minimum promoter sequence required for efficient transcription, with the blue G being the first base incorporated into the resulting RNA. The final 4 bases (GAGA) are not necessary, but many templates will show enhanced efficiency and yield if those extra bases are present.

Double-Stranded Transcription
Whilst most users will require single-stranded RNA, the T7 RNA Polymerase can be used to make double-stranded RNA for restriction into siRNA. To achieve this, the DNA template must be flanked by opposing T7 promoters. This can be obtained by including the T7 promoter in both the forward and reverse primer. The resulting transcripts can be annealed to each other by heating to 65˚C for 5 minutes and cooling slowly.

T7 Transcription Kit

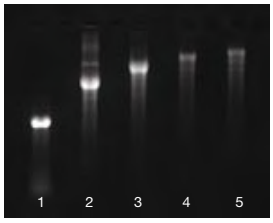
Fast and efficient transcription

FEATURES

- Fast and efficient RNA transcription from any DNA template containing a T7 promoter
- Convenient, reliable, cost-effective

APPLICATIONS

- Production of RNA and double-stranded RNA for siRNA experiments
- Generation of radioactive RNA probes with high specific activity
- Synthesis of RNA for RNA-protein interaction, splicing and processing studies
- RNA production for *in vitro* translation



PCR products of different lengths *in vitro* transcribed using the T7 Transcription Kit.
Lane 1. 1Kb RNA product
Lane 2. 3Kb RNA product
Lane 3. 5Kb RNA product
Lane 4. 7Kb RNA product
Lane 5. 9Kb RNA product

PACK SIZE	CAT NO.
50 Reactions	BIO-21072

The T7 Transcription Kit contains all the necessary components to carry out fast and efficient transcription of RNA from any DNA with a T7 promoter sequence. Moreover, it can be used to produce RNA from cloned inserts. The enzyme will incorporate labeled NTPs, and products of this enzyme can be used as probes for screening purposes.

Reaction Conditions

	20µl reaction	1ml reaction
5x Ultimate Reaction Buffer	4µl	200µl
DNA Template (0.3µg/µl)	1.0µl	(1.2 µg/µl) 25µl
DTT 1M	0.8µl	40µl
T7RNA Polymerase	0.7µl	34µl
RNase Inhibitor	0.4µl	20µl
NTP Mix, 100mM	0.8µl	40µl
DEPC-treated Water	up to 20µl	up to 1000µl

Reactions should be incubated between 37-42˚C for 2-4 hours dependent upon fragment size. This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

NTPs

Enzymatically synthesized for premium quality

FEATURES

- 98% pure by HPLC
- Convenient ready-to-use solutions
- Validated for *in vitro* transcription
- DNase, RNase and Nickase free
- Manufactured by Bioline in a purpose-built laboratory
- Custom, Bulk & OEM Nucleotide Services

APPLICATIONS

- *in vitro* transcription reactions
- Production of RNA probes and transcripts

PRODUCT	PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
NTP Set	4 x 25µmol	100mM	4 x 250µl	BIO-39052
NTP Mix	100µmol	100mM	1ml	BIO-39050

Bioline ultra-pure NTPs (Ribonucleoside-5'-triphosphates) are enzymatically synthesized from premium quality raw materials, using highly specific production systems, in our purpose-built facilities. Bioline NTPs are >98% pure as analyzed by HPLC and are free of DNase, RNase, protease, phosphatase and nicking activity.

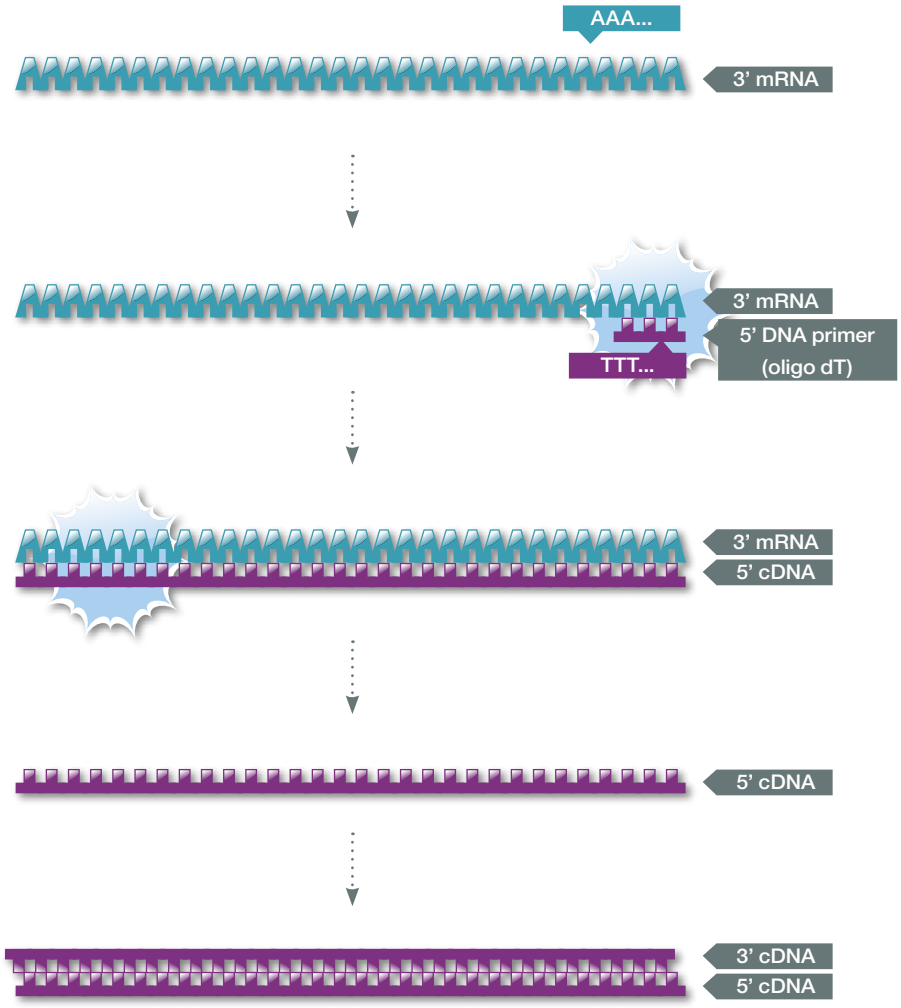
Bioline NTPs are available as a pre-optimized mix, as well as a set. The NTP Mix is a solution containing 25µmol each of ATP, GTP, CTP and UTP (pH 7.5) as sodium salts in a convenient mix at 100mM (total NTP concentration). The NTP Set consists of 4 separate 100mM solutions (ATP, GTP, CTP, and UTP, (pH 7.5)) as sodium salts. Each solution contains 25µmol (250µl) of the corresponding NTP. For *in vitro* RNA synthesis, mix equal volumes of all separate NTP solutions.

cDNA Synthesis

Process of cDNA synthesis

Reverse transcription is the process of generating DNA from an RNA template, with the help of the enzyme reverse transcriptase. The enzyme requires a primer and like other DNA polymerases, can add nucleotides only to the 3' end of the primer base-paired to the template. The new DNA is synthesized in the 5'-3' direction.

Bioline has an entire range of products for first-strand cDNA synthesis: reverse transcriptase (BioScript™), One-Step RT-PCR Kit, cDNA synthesis kit, oligo $(dT)_{18}$ and random hexamer primers and dNTPs.



Schematic representation of cDNA Synthesis

BioScript™ Moloney Murine Leukaemia Virus (MMLV) Reverse Transcriptase

CONC.	PACK SIZE	CAT NO.
200u/µl	10,000 Units	BIO-27036
200u/µl	4 x 10,000 Units	BIO-27036-4

FEATURES

Ultra-Stable Reverse Transcriptase

- **Unrivalled Stability: no loss of activity following 1 week at room temperature**
- **Working temperature range 37-50˚C**
- **Highly sensitive for enhanced cDNA yield (low RNase H activity)**
- **Produces high quality cDNA ideal for real-time PCR**
- **Ultra-pure (DNase/RNase-free)**
- **Reverse transcribes RNA templates up to 9Kb**
- **Available as part of the One-Step RT-PCR Kit**

APPLICATIONS

- **First strand cDNA synthesis for quantitative PCR**
- **cDNA library construction**
- **mRNA 5' end mapping by primer extension**
- **Dideoxynucleotide sequencing**
- **End-labeling of DNA**

BioScript™ is a Moloney Murine Leukaemia Virus (MMLV) Reverse Transcriptase, which exhibits high stability and is active at a wide range of temperatures. Unlike the wild-type enzyme, BioScript possesses low RNase H activity, which results in enhanced yields. In addition, BioScript is highly sensitive even when the amount of template is a limiting factor. BioScript is suitable for first-strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR analysis of gene expression. BioScript can be used with total RNA, mRNA or *in vitro* transcribed RNA.

PROTOCOL FOR FIRST STRAND cDNA SYNTHESIS WITH BioScript

1. Assemble the following components on ice in a certified RNase-free reaction tube:
   i. Template RNA:
   - Total RNA 0.5 - 5µg
   - Or mRNA 0.01 - 0.5µg
   - Or specific RNA up to 0.5µg

   ii. Primer:
   - Oligo$(dT)_{18}$ 0.5µg
   - Or random hexamer 0.2µg
   - Or specific oligo 5-20pmol

   iii. Deionized water (nuclease-free) up to 12µl total
2. Incubate the mix for 5 min at 70˚C, and then chill on ice.
3. Add the following components on ice:
   i. Template RNA
   ii. 10mM dNTP mix (10mM each) 1µl
   iii. 5x Reaction buffer (provided) 4µl
   iv. Deionized water (nuclease-free) up to 19.75µl total when using BioScript at 50u/reaction
4. Mix by pipetting. Add between 0.25-1.00µl of BioScript at 200u/µl.
5. Incubate at 37-45˚C for 60 min.
6. Stop the reaction by heating at 70˚C for 10 minutes, or by adding of an equal volume of 10mM EDTA (pH 7.0). Chill on ice.
7. Use the cDNA synthesized in subsequent amplification reactions without any additional purification.

General Considerations:

1. Template Quality

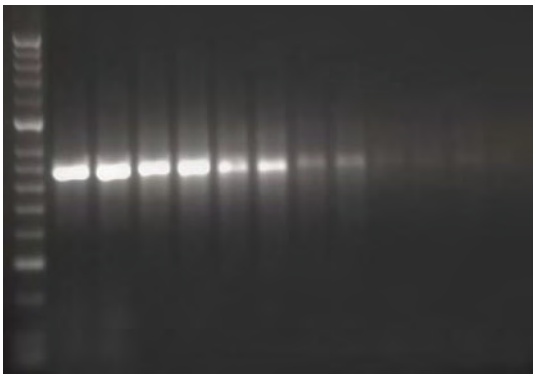
- Intact, high-quality RNA is essential for the reverse transcription reaction
- All reagents for use with RNA must be prepared using DEPC-treated Water
- The inclusion of an RNase Inhibitor protein can reduce template degradation and increase yield of PCR product.
- Low copy number genes may require an increase in starting material
- It is necessary to use a suitable RNA extraction reagent e.g., TRIsure™ (BIO-38032)

2. Extension temperature

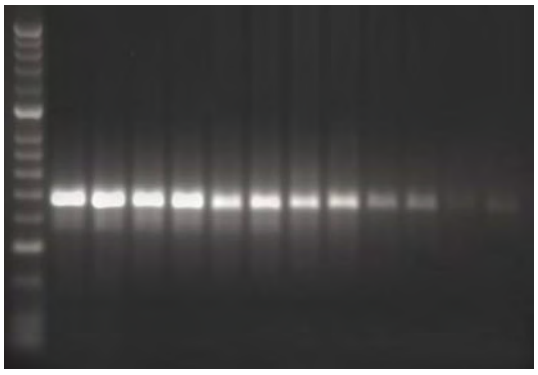
- If random hexamer oligos are used, an initial 10-minute incubation at 25˚C is recommended
- Efficient reverse transcription can be achieved at temperatures of 37˚C to 45˚C for 15-30 minutes. We recommend that initial reverse transcription steps are carried out for 30 minutes at 42˚C
- The use of higher incubation temperatures up to 50˚C may increase the yield of cDNA synthesized in cases of complex RNA secondary structure. However, the yield of the majority of RNA molecules will be reduced

cDNA SYNTHESIS TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION(S)
No cDNA synthesis	RNA Degraded	Analyze RNA on a denaturing gel to verify integrity Ensure that all reagents are RNase-free
	RNA contained an RT inhibitor	The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step
	Reaction temperature not optimal	Perform a temperature-gradient experiment
	Not enough starting RNA	Increase the amount of starting RNA, this can be an important factor when amplifying low-copy genes from total RNA
	RNA had high secondary structure	Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 60˚C (for short amplicons)
	Target not expressed in tissue analyzed	Try a different target of tissue
Poor Specificity	Non-specific annealing of primers to template	Use gene-specific primers rather than Oligo dT or random hexamers Increase the annealing temperature Increase the Tm of the primers Check for presence of pseudogenes Set up reactions on ice
	Primer dimers	Redesign primers to prevent self-annealing
	Genomic DNA contamination	Try a different target of tissue
Product in no-RTase control	Template contaminated with DNA	Treat samples with DNase I



Reverse transcription from Mouse Total RNA using BioScript.
A ten-fold serial dilution of Elite Mouse NIH3T3 Total RNA (1µg to 100pg) was reverse transcribed using 50 Units of BioScript™ and oligo $(dT)_{18}$ in a 20µl reaction volume. The resultant cDNA was then used as template in a PCR using primers for amplification of a 700bp fragment from mouse β-actin. PCR was performed using ImmoMix™ in a 50µl reaction. Reactions were carried out in duplicate. Marker is HyperLadder™ II.



High sensitivity of BioScript reverse transcriptase.
A ten-fold serial dilution of Elite Human HeLa Total RNA (1µg to 10pg) was reverse transcribed using 50 Units of BioScript™ and oligo $(dT)_{18}$ in a 20µl reaction volume. The resultant cDNA was then used as a template in a PCR using primers for amplification of a 470bp fragment from human GAPDH. PCR was performed using ImmoMix™ in a 50µl reaction. Reactions were carried out in duplicate. Marker is HyperLadder™ II.

BioScript™ One-Step RT-PCR Kit

Designed for highly sensitive one-step RT-PCR

PACK SIZE	CAT NO.
10 Reactions	BIO-65033
25 Reactions	BIO-65030
100 Reactions	BIO-65031

FEATURES

- **Simple to use, one-step set-up**
- **2x RT-PCR Mix optimized for as little as 100pg Total RNA**
- **Contains a highly sensitive blend of BioScript™ reverse transcriptase and IMMOLASE™ hot-start polymerase**
- **Supplied with a dNTP containing buffer**
- **Highly optimized for outstanding results**

APPLICATIONS

- **Gene expression analysis**
- **Gene cloning**

BioScript™ One-Step RT-PCR Kit has been designed for highly sensitive one-step RT-PCR reactions using any RNA template. The kit employs an enzyme formulation, which includes IMMOLASE™ (our widely used hot-start DNA polymerase) and BioScript, our ultra-stable reverse transcriptase. BioScript is highly sensitive even when the amount of template is a limiting factor.

The kit provides highly specific reverse transcription and PCR in a single tube, using gene-specific primers on either total RNA or mRNA. The kit is provided with RNase Inhibitor to protect template RNA from degradation. The proprietary buffer is highly optimized and balanced, leading to outstanding results.

The kit is ideal for the synthesis of double-stranded cDNA products for subsequent cloning, sequencing, expression, or transcription analysis.

The kit can be used with starting amounts of RNA template from 100pg to 2µg. After cDNA synthesis has been performed, the reaction is heated to 95˚C for 10 minutes to inactivate BioScript, and simultaneously to activate the hot-start DNA polymerase IMMOLASE (included). IMMOLASE improves specificity by eliminating the presence of non-specifics, primer-dimers, and mis-primed products.

BioScript™ One-Step RT-PCR Kit Components

COMPONENT	10 REACTIONS	25 REACTIONS	100 REACTIONS
Enzyme Mix	20µl	50µl	200µl
2x One-Step RT-PCR Reaction Buffer	250µl	625µl	2 x 1.25ml
RiboSafe RNase Inhibitor (10u/µl)	10µl	25µl	100µl
50mM $MgCl_2$ Solution	1.2ml	1.2ml	1.2ml
DEPC-treated Water	1.2ml	1.2ml	1.2ml

BioScript ONE-STEP RT-PCR KIT REACTION GUIDELINES

Template Quality

- Intact, high-quality RNA is essential for the reverse transcription reaction
- All reagents for use with RNA must be prepared using DEPC-treated Water
- The inclusion of an RNase Inhibitor protein can reduce template degradation and increase yield of PCR product
- Low copy number genes may require an increase in starting material
- It is necessary to use a suitable RNA extraction reagent e.g., TRIsure™ (BIO-38032)

$MgCl_2$ Optimization

- The final reaction will contain 1.5mM $MgCl_2$ (the 2x One-Step RT-PCR buffer contains 3mM $MgCl_2$), which should be optimal for most reverse transcriptase and PCR reactions
- $MgCl_2$ requirements for the reaction can vary, depending on the particular template and primers used
- A titration of $MgCl_2$ can be performed to optimize the reaction conditions

Primer Design and Concentration

- The use of gene-specific primers is recommended with the BioScript One-Step RT-PCR Kit. The use of oligo dT or random hexamers is not recommended with a one-step RT-PCR set-up since this can result in the generation of non-specific products
- In most cases a final primer concentration of 200nM is sufficient. However, we recommend a primer titration within the 50-500nM range
- Primers should be checked to ensure that they are not self-complementary
- Primer design can benefit from the use of an RNA secondary structure prediction model (e.g. MFOLD), to ensure that priming is not prevented by internal double-stranded regions caused by folding
- The use of intron-spanning primers allows differentiation between amplified cDNA and contaminating genomic DNA
- Annealing temperature of primers is usually melting temperature (Tm) minus 5-10˚C

Reaction Recommendations

- The use of RNase-free plasticware and tips is essential
- We recommend using a final volume of 50µl
- Prepare all reactions on ice
- Since IMMOLASE is a heat-activated enzyme, an activation period of 10 minutes at 95˚C is required prior to the cycling steps
- Efficient reverse transcription can be achieved at temperatures of 37˚C to 45˚C for 15-30 minutes. We recommend that initial reverse transcription steps are carried out for 30 minutes at 42˚C
- The use of higher incubation temperatures up to 50˚C may increase the yield of cDNA synthesized in cases of complex RNA secondary structure. However, the yield of the majority of RNA molecules will be reduced

BioScript ONE-STEP RT-PCR PROTOCOL

1. Assemble the following components on ice in a certified RNase-free reaction tube:

COMPONENT	VOLUME (µl)	FINAL CONCENTRATION
2x One-Step RT-PCR Buffer (supplied)	25	1x
One-Step Enzyme Mix (supplied)	2	-
Forward Primer (5µM)	2	200nM
Reverse Primer (5µM)	2	200nM
RNA Sample	1-10	User-determined (100pg-2µg recommended)
RNase Inhibitor (supplied)	1	10 Units
$MgCl_2$ (supplied)	2x Reaction Buffer contains 3mM $MgCl_2$. However additional Mg^{2+} may be required (see reaction guidelines)	1.5mM (Unless adjusted by the user)
DEPC-treated Water (supplied)	Up to final volume of 50µl	-
Total Volume 50µl		

2. Program the Thermal Cycler to include the RT and subsequent PCR step:

1 cycle of:

TEMPERATURE	DURATION	COMMENTS
37-45˚C	15-30 minutes	We recommend that initial reverse transcription steps are carried out for 30 minutes at 42˚C (see reaction guidelines)
95˚C	10 minutes	To denature RT enzyme and activate DNA Polymerase

Followed by 40 cycles of:

TEMPERATURE	DURATION	COMMENTS
95˚C	30 seconds	Template denaturation
50-60˚C	30 seconds	Primer annealing (actual temperature determined by primer sequence, see guidelines)
72˚C	15-30 seconds per kilobase	Extension step

3. Mix reactions gently, load into thermal cycler and start reaction.

4. Analyze the amplified product.

RT-PCR TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION(S)
No cDNA synthesis	RNA Degraded	Analyze RNA on a denaturing gel to verify integrity Ensure that all reagents are RNase-free
	RNA contained an RT inhibitor	The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step
	Reaction temperature not optimal	Perform a temperature-gradient experiment
	Not enough starting RNA	Increase the amount of starting RNA; this can be an important factor when amplifying low-copy genes from total RNA
	RNA had high secondary structure	Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 60˚C (for short amplicons)
	Target not expressed in tissue analyzed	Try a different target of tissue
Poor Specificity	Non-specific annealing of primers to template	Use gene-specific primers rather than Oligo dT or random hexamers Increase the annealing temperature Increase the Tm of the primers Check for presence of pseudogenes Set up reactions on ice
	Primer dimers	Redesign primers to prevent self-annealing
	Genomic DNA contamination	Try a different target of tissue

Suggested Controls

1. Control for DNA contamination (no RT control): Set up a standard BioScript One-Step reaction without the addition of BioScript and start at the 10 min 95°C step followed by normal PCR cycling for 40 cycles. Analyze by agarose gel electrophoresis. Presence of a product suggests DNA contamination. Treat the samples with DNase I.

2. Control for cDNA synthesis: Remove the sample from the thermal cycler after the 15-30 minutes at 37°C to 45°C step, and analyze by agarose gel electrophoresis to confirm synthesis.

3. Positive control for PCR amplification: Use a genomic template DNA (not provided) to set up a standard reaction starting at 10 min 95°C followed by normal PCR cycling for 40 cycles.

cDNA Synthesis Kit

cDNA from an RNA template

FEATURES

- Generate high quality cDNA for any downstream application
- Highly suited to low abundance total RNA ≥100pg
- Convenient, reliable, cost-effective
- Reverse transcribes RNA templates up to 9Kb

APPLICATIONS

- 1st strand cDNA synthesis for subsequent quantitative PCR
- Construction of cDNA libraries
- 2-step RT-PCR assays
- Generation of probes for hybridization
- Gene cloning

PACK SIZE	CAT NO.
30 Reactions	BIO-65025
100 Reactions	BIO-65026

The cDNA Synthesis Kit contains all necessary components to generate cDNA from an RNA template. The generated cDNA is suitable for PCR with gene-specific primers or for other downstream applications. The kit contains MMLV reverse transcriptase and is suitable for first strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR amplifications. The cDNA Synthesis Kit is optimized for RT reactions using a wide range of total RNA amounts (100pg-2µg), such that long and low abundance cDNAs can be detected by amplification after cDNA synthesis. The kit contains oligo $(dT)_{18}$ and random hexamer primers together with control RNA template. The dNTPs included in the kit are manufactured by Bioline and are 99% pure.

The cDNA Synthesis Kit contains enough reagents for either 30 or 100 single-strand reactions.

cDNA Synthesis Kit Components

COMPONENT	30 REACTIONS	100 REACTIONS
5x RT Buffer	120µl	400µl
(200u/µl) Reverse Transcriptase	7.5µl	25µl
(10u/µl) RNase Inhibitor	30µl	100µl
dNTP Mix 10mM Total	30µl	100µl
Oligos $(dT)_{18}$ Primer Mix	30µl	100µl
Random Hexamer Primer Mix	30µl	100µl
Control RNA Template 1µg/µl (enough for 5 reactions)	5µl	5µl
DEPC-treated Water	1.2ml	1.2ml

cDNA Synthesis Kit Reaction Guidelines

Template Quality

- Intact, high-quality RNA is essential for the reverse transcription reaction
- All reagents for use with RNA must be prepared using DEPC-treated Water
- The inclusion of an RNase Inhibitor protein can reduce template degradation and increase yield of PCR product
- Low copy number genes may require an increase in starting material
- Use a suitable RNA extraction reagent e.g. TRIsure™ (BIO-38032)

Primer Design and Concentration

There are three methods for priming cDNA synthesis:

- **Oligo dT**
  Oligo dT priming uses the poly-A tail found on the 3' end of most eukaryotic mRNAs. This ensures that the 3' end of mRNAs are represented, although long mRNAs can have their 5' ends under-represented in the subsequent cDNA pool (use at 50pmoles/reaction).

- **Random Primers**
  Random priming gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Random priming is unable to distinguish between mRNA and other RNA species present in the reaction (Use at 50-250ng/reaction).

- **Gene Specific Primers**
  Gene specific primers are designed to generate cDNA for a specific gene of interest. It is a widely used method for performing one-step RT-PCR when only 1 gene is under investigation. It can be useful when RNA concentrations are low (Use at 10-20pmol/reaction).

For most applications, Oligo dT priming is recommended.

Reaction Recommendations

- The use of RNase-free plasticware and tips is essential
- We recommend using a final volume of 50µl
- Prepare all reactions on ice
- Efficient reverse transcription can be achieved at temperatures of 37˚C to 45˚C for 15-30 minutes. We recommend that initial reverse transcription steps are carried out for 30 minutes at 42˚C
- The use of higher incubation temperatures up to 50˚C may increase the yield of cDNA synthesized in cases of complex RNA secondary structure. However, the yield of the majority of RNA molecules will be reduced

cDNA SYNTHESIS KIT PROTOCOL

1. Prepare the following on ice:

(1µg) RNA	nµl
Oligo $(dT)_{18}$ or Random Hexamer	1µl
10mM dNTP	1µl
DEPC-treated Water	up to 10µl

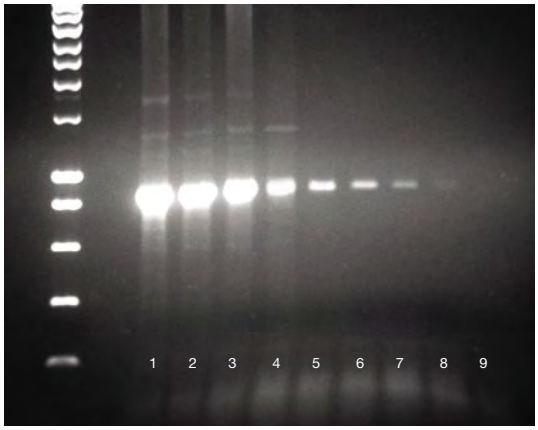
2. Incubate samples at 65˚C for 10 minutes.
3. Place on ice for 2 minutes.
4. Prepare the following reaction mix:

COMPONENT	1 REACTION	10 REACTIONS
5x RT Buffer	4µl	40µl
RNase Inhibitor (10u/µl)	1µl	10µl
Reverse Transcriptase (200u/µl)	0.25µl	2.5µl
DEPC-treated Water	to 10µl	to 100µl

5. Add 10µl of the above reaction mix to a tube containing the primed RNA.
6. Incubate samples at between 37-45˚C for 30-60 minutes.
7. Terminate reaction by incubating at 70˚C for 15 minutes, chill on ice.
   These data are intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

cDNA SYNTHESIS KIT TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION(S)
No cDNA synthesis	RNA Degraded	Analyze RNA on a denaturing gel to verify integrity Ensure that all reagents are RNase-free
	RNA contained an RT inhibitor	The presence of inhibitors can be determined by mixing a control RNA with some of the sample and comparing the yield with that of the original amplification. Remove inhibitors such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step
	Reaction temperature not optimal	Perform a temperature-gradient experiment
	Not enough starting RNA	Increase the amount of starting RNA, this can be an important factor when amplifying low-copy genes from total RNA
	RNA had high secondary structure	Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 60˚C (for short amplicons)
	Target not expressed in tissue analysed	Try a different target of tissue
Poor Specificity	Non-specific annealing of primers to template	Use gene-specific primers rather than Oligo dT or random hexamers Increase the annealing temperature Increase the Tm of the primers Check for presence of pseudogenes Set up reactions on ice
	Primer dimers	Redesign primers to prevent self-annealing
	Genomic DNA contamination	Try a different target of tissue
Product in no-RTase control	Template contaminated with DNA	Treat samples with DNase I



Reverse Transcription using cDNA Synthesis Kit.
High sensitivity was observed with a serial dilution experiment: Various quantities of total HeLa RNA were reverse transcribed using the cDNA Synthesis Kit in a 20µl reaction. Oligo$(dT)_{18}$ was used as primer. Subsequently, 5µl of each reaction was used in conjunction with β-actin specific primers to amplify an 860bp band from human mRNA.
Lanes: 50ng (1), 25ng (2), 10ng (3), 1ng (4), 500pg (5), 250pg (6), 100pg (7), 50pg (8) and 0pg (9). Marker is HyperLadder™ I.

CellSure cDNA Kit

cDNA directly from cultured cells for analysis by PCR

PACK SIZE	CAT NO.
30 Reactions	BIO-65040
100 Reactions	BIO-65041

FEATURES

- **Quick and easy production of cDNA directly from cell culture**
- **No RNA extraction required**
- **From cells to cDNA in 90 minutes**

APPLICATIONS

- **The cDNA is suitable for standard or real-time PCR assays**
- **High throughput gene expression analysis**

CellSure cDNA Kit is a convenient kit designed to quickly generate cDNA directly from cultured cells for analysis by PCR. The kit eliminates the need to purify RNA, which can be a time-consuming process and can lead to loss of sample, especially when starting material is limited. The kit is the ideal choice for researchers who wish to perform reverse transcription reactions on a small population of cells and provides sufficient cDNA for multiple PCR reactions.

A crude RNA extract is produced by a simple lysis step followed by heat treatment to inactivate RNases, and a DNase I treatment to degrade genomic DNA. The crude RNA extract is then used to synthesize cDNA using the reverse transcriptase provided. The kit can be used with a variety of mammalian cell lines including HeLa and NIH3T3.

CellSure cDNA Kit contains our reverse transcriptase, which is active over a wide range of temperatures.

CellSure cDNA Kit Components

COMPONENT	30 REACTIONS	100 REACTIONS	DESCRIPTION
Cell Lysis buffer	3ml	10ml	Lyses the cells
1x PBS (pH 7.4)	30ml	100ml	Removes serum proteins from the cells
DNase I (2u/µl)	60µl	200µl	Degrades any DNA
5x Reverse Transcriptase Buffer	300µl	1ml	Reverse Transcriptase Buffer
Reverse Transcriptase	30µl	100µl	Reverse Transcriptase
RNase inhibitor (10u/µl)	30µl	100µl	High-affinity RNase inhibitor prevents degradation of RNA template
dNTP mix (10mM)	120µl	400µl	High-purity (99%) dNTP mix manufactured by Bioline
Random Hexamers (50µM)	60µl	200µl	5' NNNNNN 3' for reverse transcription where transcripts are long or have significant secondary structure
Oligo $(dT)_{18}$ (50µM)	60µl	200µl	5' TTTTTTTTTTTTTTTTT 3' for reverse transcription where gene specific primers are designed close to the 3'
DEPC-treated Water	1.75ml	2 x 1.75ml	DEPC-treated H_2O free of detectable RNase activity
Mouse Total RNA (1µg/µl)	10µl	10µl	Control RNA
Control Primer mix (10µM)	10µl	10µl	Control Primer mix

Note: HyperLadder is a trademark of Bioline.

CellSure cDNA KIT PROTOCOL

Overview:

A cell lysis buffer is used to lyse cells, which is followed by heat treatment to inactivate RNases. Contaminating genomic DNA is degraded by incubation with DNase I. A further heat treatment step inactivates the DNase I and the lysate is ready for reverse transcription.

Cell lysis and DNase I treatment:

The following protocol is for preparing a cell lysate with 2 x 10^5 HeLa cells.

1. Count or estimate the number of cells.

   i. Adherent cells: For cells grown in a 96-well plate, ensure that the final cell concentration does not reach >10^5 cells/well, since this would result in inhibition of the RT-PCR reaction.
   For cells grown in larger cell-culture vessels, detach the cells and then count the number.

   ii. Suspension cells: Count cells directly in their growth medium.

2. Pipette 2 x 10^5 cells into a microcentrifuge tube. Centrifuge at 200 x g for 5 minutes in a bench centrifuge to pellet the cells.

3. Remove the growth medium and wash cells at least once with 500µl of cold 1x PBS. For a 96-well plate, add cold 1x PBS directly to the cells in the well and discard.

4. Centrifuge as before, discard the supernatant and place the cells on ice.

5. Resuspend the cells in 100µl ice cold Cell Lysis Buffer.

Control: For the control reaction, instead of the cells, add 1µl of Control Mouse Total RNA to 100µl of Cell lysis buffer.

Cell lysate concentrations should be 1-2000 cells per µl of lysis buffer. To ensure optimum lysis conditions, do not lyse in more than 100µl, but do not exceed 2000 cells per µl as this may inhibit the RT-PCR reaction.
For a small number of cells (≤10000 cells) or analysis of low-expressing genes, lyse the cells in a minimum of 5µl of lysis buffer. For single cell analysis, lyse in 5µl after which the whole lysate can be used for analysis by one-step RT-PCR.

6. Pipette up and down several times to mix and ensure cell disruption and leave on ice until the Cell Lysis Buffer has been added to all the samples. For a 96 well plate, lyse the cells directly in each well.

7. Incubate at 75˚C for 10 minutes to inactivate RNases and then place on ice to cool for 2 minutes.

8. Add DNase I to a final concentration of 0.04u/µl and incubate at 37˚C for 15 minutes to degrade genomic DNA.

9. Inactivate the DNase I by heating at 75˚C for 5 minutes.

10. Place on ice and the lysate is ready for RT-PCR (lysate can be stored at -20˚C at this stage).

Storage of the cell lysates:

Lysates made from ≥2.5 x 10^4 cells can be stored at -20˚C for up to 1 week, or at -80°C for up to 2 months.
Lysates made from ≤2.5 x 10^4 cells should be used for RT-PCR immediately.

Two-step RT-PCR PROTOCOL

Important Note: PCR components are not supplied with this kit.

No-RT control: Include a No-RT control reaction with all components in the mix except for the reverse transcriptase.

1. For a 20µl reverse transcription reaction, assemble the following components in a microcentrifuge tube:

COMPONENT	AMOUNT	SOURCE
Cell lysate	5-10µl	Generated by kit
Oligo dT and/or random hexamers*	2µl	Supplied
dNTPs (10mM)	1µl	Supplied
DEPC-treated Water	Up to 10µl	Supplied

** Either Oligo dT or random hexamers can be used to prime the RT reaction. Some users use a combination of Oligo dT and random hexamers in a molar ratio of 10:1 or 3:1 respectively, with a final concentration of 10µM per reaction. If a gene-specific primer is used to prime the RT reaction, its final concentration should be 0.25-5µM.*

2. Heat at 70˚C for 5 minutes.

3. Then add:

COMPONENT	AMOUNT	SOURCE
5x RT buffer	4µl	Supplied
RNase Inhibitor	1µl	Supplied
Reverse Transcriptase	0.25µl	Supplied
DEPC-treated Water	Up to 20µl	Supplied

4. Incubate at 42˚C for 30-60 minutes.

5. Heat at 70˚C for 10 minutes to inactivate the BioScript reverse transcriptase.

6. Store the RT reaction at -20˚C or proceed to the amplification step.

7. For a 50µl PCR reaction, assemble the following components on ice and mix by pipetting or gentle vortexing:

Control: For a control reaction, use the Control Primer mix with cDNA generated from the Control Mouse Total RNA lysate.

COMPONENT	AMOUNT	SOURCE
cDNA	1-5µl	Generated by kit
10x PCR buffer	5µl	Not supplied
50mM $MgCl_2$	1.5µl	Not supplied
10mM dNTP mix	4µl	Supplied
Forward primer	200-900 nM	*Control supplied
Reverse primer	200-900 nM	*Control supplied
Thermostable DNA polymerase	2 Units	Not supplied
Nuclease-free dH_2O	Up to 50µl	Not supplied

**Substitute forward and reverse primers with 1µl Control Primer mix in a 50µl PCR.*

8. Program a thermal cycler for the following PCR conditions:

TEMPERATURE	DURATION	CYCLES
94˚C	2 minutes	1
94˚C	30 sec	30-40
Annealing temperature*	30 sec	
72˚C	30 sec	
72˚C	5 minutes	1

** Start with an annealing temperature of 55˚C*

9. Analyze the amplified products.

One-step RT-PCR
The lysate can also be used directly in a one-step RT-PCR reaction. We recommend 1-5µl of lysate in a 25µl reaction volume using the One-Step RT-PCR Kit (BIO-65030).

Quantitative PCR
The lysate is also suited to RT-PCR by real-time methods including two-step and one-step with both SYBR® Green and fluorescent probes.

General Considerations:

Cell numbers
For different cell types, it may be necessary to optimize the number of cells for lysis, as there may be inhibitory factors present in your lysate that will inhibit the RT-PCR reaction.

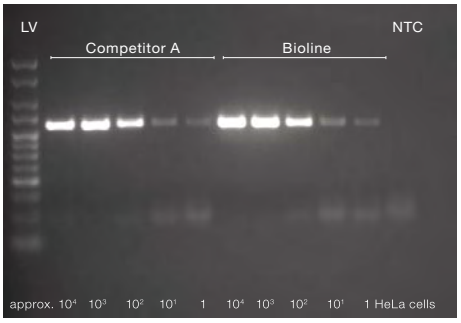
Genomic DNA contamination
If genomic DNA contamination is still evident after DNase I treatment, use twice the amount of DNase I to provide a final concentration of 0.08u/µl in the lysate, or increase the incubation time to 30 minutes.

Primer Design
When designing primers it is important for RT-PCR that the primers flank at least one intron, or that one of the primers spans an exon-exon boundary in order to prevent any contaminating genomic DNA from being amplified. If the gene of interest has processed pseudogenes that may be present in the genomic DNA, then the PCR product from both the cDNA and the genomic DNA will be the same size. Wherever possible, design primers to avoid regions of secondary structure in the mRNA.

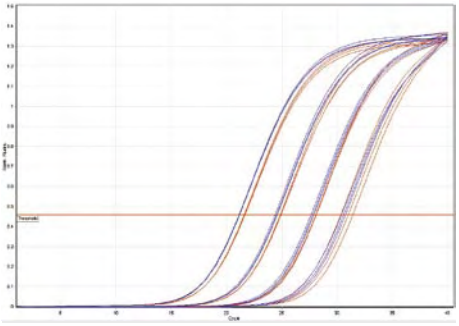
RNase Inactivation temperature
When inactivating RNases during the 10-minute incubation at 75˚C, it is important to ensure that the temperature of the samples themselves reaches 75tC. To do so, use a calibrated heating device and heat well in advance. If necessary, check the temperature of a mock reaction using a thermometer with a microprobe.

CellSure cDNA KIT TROUBLESHOOTING GUIDE

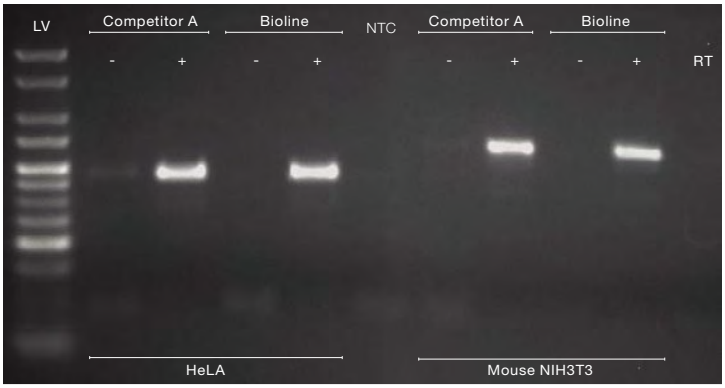
OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION(S)
No or low amounts of PCR products detected	Insufficient PCR cycles	Increase the number of cycles performed
	RNases not completely inactivated	Reduce the cell concentration in the cell lysis buffer. Ensure that in step 7 of the 'Cell lysis and DNase I treatment' section of the protocol, the cell lysate reaches 75˚C
	Cell lysate contains inhibitors of RT	Reduce the number of cells added to the cell lysis buffer
	RNA had high secondary structure	Prior to reaction set-up, denature RNA with primers. Raise the temperature of the RT step, up to a maximum of 70˚C (for short amplicons)
	RNA degradation	To prevent RNA degradation, all buffers must be kept on ice
Unspecific PCR products	Non-specific annealing of primers to template	Increase the annealing temperature
	Primer dimers	Redesign primers to prevent self-annealing
Product in no-RTase control	Template contaminated with Genomic DNA	Increase the incubation time of the DNase I treatment to 30 minutes. Increase the concentration of DNase I to 0.08u/µl



Amplification of a fragment of the GAPDH gene from cDNA obtained from HeLa cells using the CellSure cDNA Kit and Competitor A's kit.
A serial dilution of the lysate was performed prior to RNase inactivation, DNase treatment and cDNA synthesis. The approximate number of cells used per reaction is as indicated in the figure. Marker is HyperLadder™ V.



CellSure cDNA Kit in two-step RT-PCR using SYBR® Green I chemistry.
DNA synthesized from cell lysates containing approximately 625 to 0.625 cells were prepared using CellSure cDNA Kit and Competitor A's kit. The cDNA was subsequently used in qPCR reactions using SensiMix™ (Quantace) and SYBR® Green I chemistry on a Rotor-Gene™ 6000 (Corbett Life Science). A fragment of the Ubiquitin gene was amplified. Traces in red and blue correspond to cDNA obtained using the CellSure cDNA Kit kit and Competitor A's kit respectively.



Amplification of a fragment of the β-actin gene from cDNA obtained from HeLa and NIH3T3 cells using CellSure cDNA Kit & Competitor A's kit.
Approximately 2.5 x 10^3 cells were used in a 25µl PCR reaction using BIOTAQ™ DNA Polymerase. Marker is HyperLadder™ V.

Oligo $(dT)_{18}$

Primer for first strand cDNA synthesis

PACK SIZE	CAT NO.
27µg	BIO-38029

APPLICATIONS

- **cDNA synthesis with a Reverse Transcriptase**

Primer sequence
5'-d (TTT TTT TTT TTT TTT TTT)-3'

Concentration
100µl at 270ng/µl

Oligo $(dT)_{18}$ Primer is suitable for use as a primer for first strand cDNA synthesis with a reverse transcriptase. The primer hybridizes to the poly-adenylated tail found on the 3' end of most eukaryotic mRNAs. Oligo $(dT)_{18}$ ensures that the 3' end of mRNAs are represented.

Random Hexamer Primers

Oligonucleotides representing all possible hexamer sequences

PACK SIZE	CAT NO.
25µg	BIO-38028

APPLICATIONS

- **cDNA synthesis using a Reverse Transcriptase with RNA templates**
- **DNA synthesis using Klenow fragment with DNA templates**
- **DNA probe synthesis for use in Southern, Northern, and *in situ* hybridization applications**

Primer sequence
5' – d (NNNNNN) –3' N = G, A, T or C

Concentration
500µl at 50ng/µl

Random Hexamer Primers consist of a mixture of oligonucleotides representing all possible hexamer sequences. Random Hexamer Primers are commonly used for priming single-stranded DNA or RNA for extension by DNA polymerases or reverse transcriptases. During cDNA generation, random priming gives random coverage to all regions of the RNA to generate a cDNA pool containing various lengths of cDNA. Random priming is incapable of distinguishing between mRNA and other RNA species present in the reaction.

dNTPs

Enzymatically synthesized for premium quality

PRODUCT	PACK SIZE	FINAL CONC.	CAT NO.
dNTP Set	4 x 25µmol	100mM total	BIO-39025
dNTP Set	4 x 100µmol	100mM total	BIO-39026
dNTP Set	4 x 100µmol	100mM total	BIO-39049
dNTP Set	4 x 500µmol	100mM total	BIO-39027
dNTP Mix	10µmol	10mM total	BIO-39044
dNTP Mix	20µmol	40mM total	BIO-39043
dNTP Mix	50µmol	100mM total	BIO-39028
dNTP Mix	100µmol	10mM total	BIO-39053
dNTP Mix	200µmol	100mM total	BIO-39029

FEATURES

- **Ultra-pure: >99% trisphosphate by HPLC**
- **Extended shelf-life of 24 months at -20˚C**
- **Free from PCR inhibitors**
- **DNase, RNase and nickase free**
- **Manufactured by Bioline in a purpose-built facility**

APPLICATIONS

Suitable for a wide variety of applications such as:

- **Standard and long range PCR assays**
- **cDNA synthesis**
- **qPCR**
- **Microarrays**
- **DNA sequencing**
- **Labeling**

Bioline is a primary manufacturer of ultra-pure dNTPs. Manufactured in a purpose-built facility, Bioline dNTPs are enzymatically synthesized for premium quality and possess at least 99% purity by HPLC. The dNTPs are tested for the absence of DNase, RNase, protease and nickase activity. Bioline dNTPs are stable for 24 months when stored in a -20˚C constant-temperature freezer.

RNA Visualization

Once RNA has been isolated, it can be visualized by agaorse electrophoresis. It is recommended to load RNA samples with specially designed RNA loading buffers and run them with MOPS buffer. Bioline also provides RNA ladders for accurate sizing of your products.

RiboLadders

RNA molecular weight markers

PRODUCT	PRESENTATION	PACK SIZE	CAT NO.
RiboLadder Long	1 x 100µl	25 Lanes	BIO-33061
RiboLadder Short	1 x 100µl	25 Lanes	BIO-33060

FEATURES

- **Easy identification and orientation**
- **Loading Buffer included**

RiboLadder Long:

- **Band sizes from 500b to 9000b**

RiboLadder Short:

- **Band sizes from 100b to 1000b**

RNA Concentration

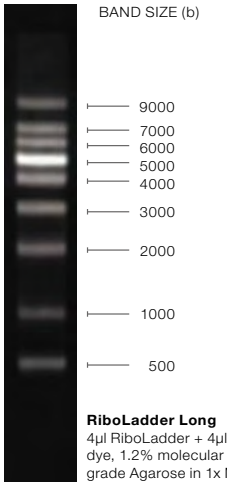
RiboLadder Long: 1.5µg/µl
RiboLadder Short: 4.5µg/µl
For optimum resolution we recommend 4µl of RiboLadder Long or RiboLadder Short per lane.

RiboLadder Long is a single-stranded RNA molecular weight marker with band sizes ranging from 500b to 9000b. For easy identification and orientation, a band at 5000b has the highest intensity. RiboLadder Short is a single-stranded RNA molecular weight marker with band sizes ranging from 100b to 1000b. For easy identification and orientation, a band at 500b has the highest intensity.

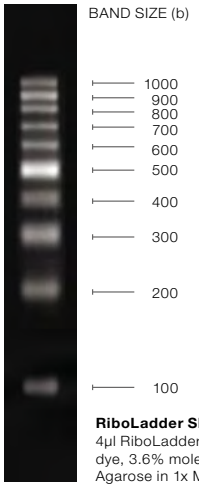
The ladder sequences are derived from transcribed lambda sequences using *in vitro* transcription. RiboLadders contain 20% formamide.

2x RNA Loading Buffer with ethidium bromide is supplied for loading both RiboLadder and samples. The inclusion of ethidium bromide makes it unnecessary to add this to the gel.

If the RNA is to be used in a Northern Blot, we recommend using 2x RNA Loading Buffer without ethidium bromide. Ethidium bromide reduces hybridization efficiency once that the RNA is transferred to a membrane.



RiboLadder Long
4µl RiboLadder + 4µl RNA loading dye, 1.2% molecular biology grade Agarose in 1x MOPS



RiboLadder Short
4µl RiboLadder + 4µl RNA loading dye, 3.6% molecular biology grade Agarose in 1x MOPS

MOPS-EDTA-Na Acetate Buffer

Designed for agarose gel electrophoresis of RNA

FEATURE
- DNase/RNase-free

APPLICATION
- Agarose gel electrophoresis of RNA

PACK SIZE	CAT NO.
1 Litre	BIO-38027

MOPS (3-Morpholinopropanesulfonic acid)-EDTA-sodium acetate buffer is especially designed for agarose gel electrophoresis of RNA. The buffer is supplied at a 10x concentration and is ready for use. For optimal denaturation and visualization of samples we recommend Bioline RNA loading buffers.

RNA Loading buffers

Designed for loading RNA samples

FEATURES
- Available with or without ethidium bromide
- Ready-to-use solution

APPLICATIONS
- Agarose gel electrophoresis of RNA samples
- Northern Blot analysis

PRODUCT	CONC.	PACK SIZE	CAT NO.
RNA Loading Buffer with Ethidium Bromide	2x	1ml	BIO-38025
RNA Loading Buffer without Ethidium Bromide	2x	1ml	BIO-38026

The 2x RNA loading buffers maintain the denatured state of the RNA during electrophoresis. The ready-to-use buffer solutions are available either with or without ethidium bromide. We recommend using RNA loading buffer on MOPS agarose gels. The buffer can also be used on formaldehyde, glyoxal, agarose gels and acrylamide urea gels.

Agarose

DNase/RNase-free

FEATURES
- DNase/RNase-free
- Excellent value and purity
- High gel strength (>1500g/cm^2)
- Available as powder or as pre-weighed 0.5g tablets

PRODUCT	PACK SIZE	CAT NO.
Agarose HiRes	100g	BIO-41029
Agarose	100g	BIO-41026
Agarose	500g	BIO-41025

Bioline Agarose (DNase/RNase-free) is an extremely pure, high molecular biology grade agarose powder that has been extensively tested for DNase and RNase contamination. Bioline Agarose provides high resolution of DNA and RNA fragments of sizes >1000bp and offers consistent resolution from lot to lot.

The high gel strength (>1500g/cm2) means that gels as low as 0.5% are feasible. Also available is Bioline Agarose HiRes, an excellent quality agarose specially designed for the resolution of nucleic acid fragments smaller than 1000bp.

Agarose Tablets

Bioline "pre-weighed" Agarose

Bioline "pre-weighed" Agarose Tablets (DNase/RNase-free) are designed to provide a cleaner, safer, no-mess environment, and more convenience than powdered agarose. Each tablet contains a pre-determined amount of agarose (0.5g), eliminating the need to weigh out loose agarose powder. Simply add the appropriate number of tablets to your buffer, incubate at room temperature for five minutes and prepare your gel as normal.

PACK SIZE	CAT NO.
150g	BIO-41028
300g	BIO-41027

RNA Templates and Controls

Using the appropriate controls is extremely essential in any assay. Here, we present ready-to-use, validated total RNA from different sources for use in a wide range of applications.

Elite Human HEK293 Total RNA

RNA is isolated from the human embryonic kidney

FEATURES
- Ready-to-use validated total RNA
- DNase I treated to minimize genomic DNA contamination

APPLICATIONS
- Suitable template for use in RT-PCR, Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation

Concentration
1µg/µl

PACK SIZE	CAT NO.
100µg	BIO-38034

Elite Human HEK293 Total RNA is isolated from the human embryonic kidney epithelial cell line; HEK293, using TRIsure™ (BIO-38032). The RNA is DNase I treated, precisely quantified and resuspended in 1mM sodium citrate pH 6.4 at 1mg/ml. Elite Total RNA is a suitable template for cDNA synthesis as well as for one- and two-step qualitative and quantitative RT-PCR assays. Elite Total RNA can also be used as a template for Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation.

Elite Human HeLa Total RNA

RNA is isolated from the human cervical

FEATURES
- Ready-to-use validated total RNA
- DNase I treated to minimize genomic DNA contamination

APPLICATIONS
- Suitable template for use in RT-PCR, Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation

Concentration
1µg/µl

PACK SIZE	CAT NO.
100µg	BIO-38035

Elite Human HeLa Total RNA is isolated from the human cervical epithelial adenocarcinoma cell line; HeLa, using TRIsure™ (BIO-38032). The RNA is DNase I treated, precisely quantified and resuspended in 1mM sodium citrate pH 6.4 at 1mg/ml. Elite Total RNA is a suitable template for cDNA synthesis as well as for one and two-step qualitative and quantitative RT-PCR assays. Elite Total RNA can also be used as a template for Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation.

Elite Mouse NIH3T3 Total RNA

RNA is isolated from the mouse

FEATURES
- Ready-to-use validated total RNA
- DNase I treated to minimize genomic DNA contamination

APPLICATIONS
- Suitable template for use in RT-PCR, Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation

Concentration
1µg/µl

PACK SIZE	CAT NO.
100µg	BIO-38036

Elite Mouse NIH3T3 Total RNA is isolated from the mouse embryonic fibroblast cell line, NIH3T3; using TRIsure™ (BIO-38032). The RNA is DNase I treated, precisely quantified and resuspended in 1mM sodium citrate pH 6.4 at 1mg/ml. Elite Total RNA is a suitable template for cDNA synthesis as well as for one- and two-step qualitative and quantitative RT-PCR assays. Elite Total RNA can also be used as a template for Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation.

Associated products

After cDNA synthesis, co-precipitants can be used to ensure maximum recovery of nucleic acids. For purification or concentration of your products, Bioline recommends SureClean. These products are described below, along with other associated products which could facilitate your work with RNA.

RiboSafe RNase Inhibitor

Complete inhibition of RNases

CONC.	PACK SIZE	CAT NO.
40u/µl	2500 Units	BIO-65027
40u/µl	10000 Units	BIO-65028

FEATURES

- **Complete inhibition of RNase A, B and C**
- **DNase/RNase and Nickase-free**
- **No inhibition of polymerase/transcriptase activity**
- **Stable over a wide range of pH, DTT concentrations and temperatures**

APPLICATIONS

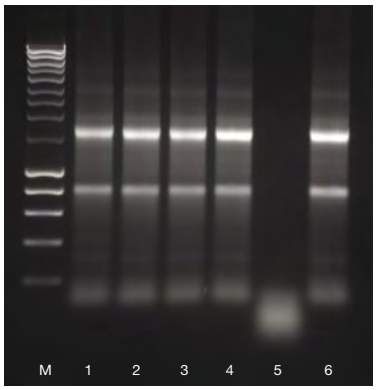
- **RNA purification**
- **cDNA preparation by reverse transcription**
- **RNA sequencing**
- ***in vitro* RNA transcription**
- ***in vitro* protein synthesis**

RiboSafe Ribonuclease Inhibitor is a recombinant mammalian protein which completely inhibits a broad spectrum of eukaryotic RNases, including RNase A, B and C by binding non-covalently in a 1:1 ratio, with an association constant of 10^{14}.

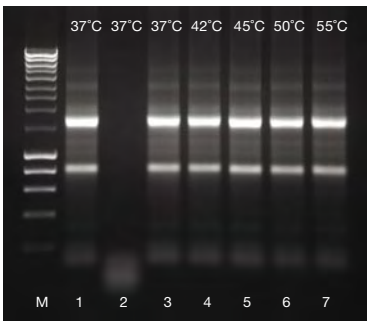
RiboSafe is active over a broad range of reaction conditions - a wide range of pH, DTT concentrations and temperatures - allowing greater flexibility in experimental design.

RiboSafe is useful in any applications where the presence of RNases is a potential problem. By protecting the RNA from degradation, RiboSafe can significantly increase the detection limits of RT-PCR. It does not show inhibition of polymerase or transcriptase activity.

RiboSafe RNase Inhibitor is tested for activity, SDS-PAGE purity, and the absence of endonucleases, nickases and exonucleases.



RiboSafe inhibits increasing amounts of RNase A with high efficiency
2µg of total human HeLa cell RNA was incubated with 20 Units of RiboSafe RNase Inhibitor and 2ng, 750pg, 250pg and 125pg of RNase A (lanes 1-4) at 37°C for 30 min. Controls were total HeLa cell RNA (lane 6) and 2µg of total human HeLa cell RNA incubated with 125pg RNase A and no RiboSafe RNase Inhibitor (lane 5). Marker is HyperLadder™ I.



Thermostability of RiboSafe RNase Inhibitor
2µg of total human HeLa cell RNA was incubated with 20 Units of RiboSafe RNase Inhibitor and 125pg of RNase A for 30 minutes at increasing temperatures (lanes 3-7). Control reaction was total human HeLa cell RNA incubated at 37°C without RNase Inhibitor (lane 1) and non-template control (lane 2). Marker is HyperLadder™ I.

Co-Precipitants

For maximum recovery of nucleic acids

Bioline Co-Precipitants are linear polyacrylamides that aid salt/alcohol precipitation of DNA and RNA. Co-Precipitants are free from contamination and are suitable for most applications, including the precipitation of DNA for sequencing, after enzymatic manipulations and RNA from different sources. Recovery is close to 100% and the products are suitable for sequencing or other sensitive applications. Available with and without dye. Glycogen is also available as a co-precipitant.

PRODUCT	CONC.	PACK SIZE	CAT NO.
Co-Precipitant Colorless	5mg/ml	1.5ml	BIO-37074
Co-Precipitant Pink	5mg/ml	1.5ml	BIO-37075
Glycogen	20mg/ml	1ml	BIO-37077

DEPC-treated Water

High-quality molecular grade water

Bioline DEPC-treated Water is ready-to-use, DNase/RNase-free, ultra-pure, high-quality molecular grade water ideal for use in all RNA work.

DEPC-treated Water is prepared by treating ultra-pure 18.2MΩ Water with diethylpyrocarbonate (DEPC), and is then autoclaved to inactivate the DEPC.

PACK SIZE	CAT NO.
10 x 10ml	BIO-38030
1 Litre	BIO-38031

SureClean

Column-free method for nucleic acid purification

FEATURES

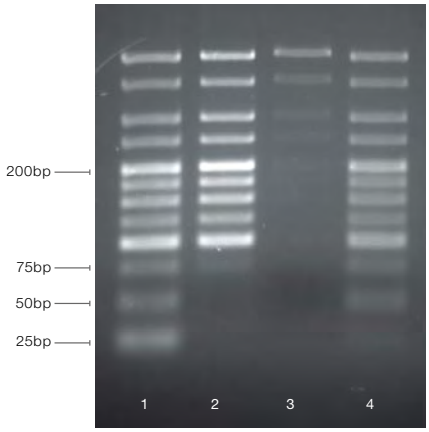
- **Column-free PCR clean-up**
- **Post-PCR recovery of up to 98%**
- **Cost-effective, simple and rapid protocol**
- **Products are suitable for immediate downstream applications**
- **Also available with pink dye for improved visibility & minimal pellet loss (SureClean Plus)**

APPLICATIONS

- **PCR clean-up**
- **Removes primers, primer-dimers, dNTPs and restriction enzymes**
- **DNA or dsRNA purification or concentration**

SureClean is a novel, inexpensive solution, which provides a column-free method for nucleic acid purification. Using a simple and rapid procedure, SureClean can be used to purify or concentrate DNA or dsRNA from PCR reactions or any enzymatic digests. This method is easy to follow, combining convenience, speed and excellent recovery rates. A version of SureClean known as SureClean Plus incorporates a pink co-precipitant that facilitates easy visualization of the purified pellet.

PRODUCT	PACK SIZE	CAT NO.
SureClean	1 x 5ml	BIO-37042
SureClean	1 x 25ml	BIO-37046
SureClean Plus	1 x 5ml	BIO-37047
SureClean Plus	1 x 25ml	BIO-37048



DNA purification using SureClean and Competitor Q's & X's spin-column purification methods.
30µl of HyperLadder™ V was purified using the manufacturer's protocols. For each of the methods DNA was resuspended in 30µl TE, of which 5µl was loaded on to a 3.5% agarose gel.
Lane 1. HyperLadder™ V
Lane 2. HyperLadder™ V purified using SureClean
Lane 3. HyperLadder™ V purified using spin-columns from Competitor X
Lane 4. HyperLadder™ V purified using spin-colums from Competitor Q

Note: HyperLadder is a trademark of Bioline.

For the latest in product information and technical support please visit www.bioline.com

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info@bioline.com
www.bioline.com

United Kingdom

Bioline
16 The Edge Business Centre
Humber Road
London NW2 6EW
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

Germany

Bioline GmbH
Im Biotechnologiepark, TGZ 2
D-14943 Luckenwalde
Tel: +49 (0)3371 68 12 29
Fax: +49 (0)3371 68 12 44

Australia

Bioline (Aust) Pty Ltd
PO Box 122
Alexandria NSW 1435
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

USA

Bioline USA Inc.
305 Constitution Drive
Taunton MA 02780
Tel: 508-880-8990
Fax: 508-880-8993
Order Toll Free: 1-888-257-5155