3rd 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.

How to maintain an RNase-free environment

For correct storage of RNA it is 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.

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.

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.

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.

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.

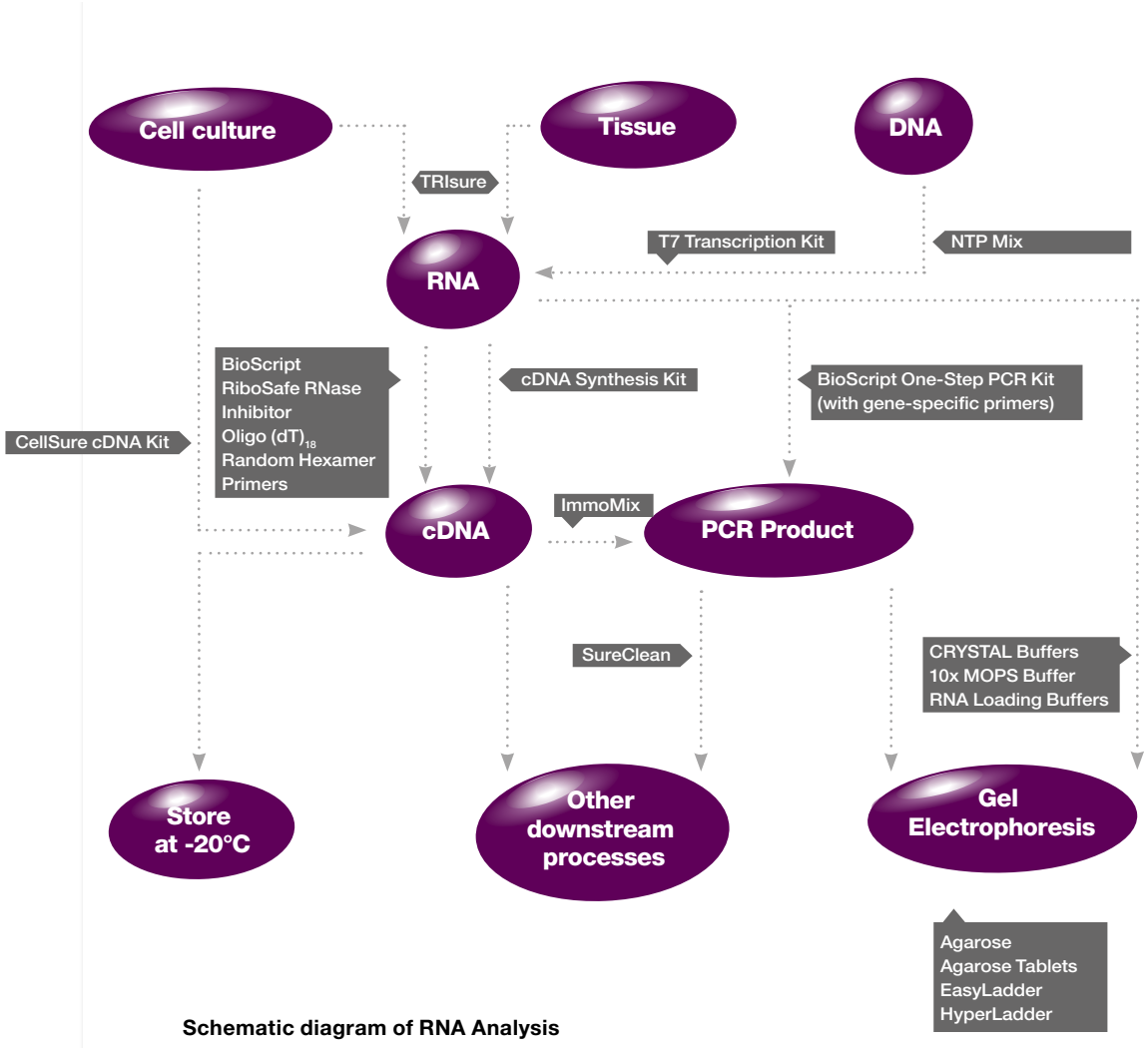
The most common procedure for determining RNA integrity is running 2-4µg of a total RNA sample on an agarose denaturing gel. The RNA may be visualized by EtBr staining, which reveals the ribosomal RNA bands. These bands can vary depending on the organism the RNA was extracted from (see table). In general, 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 grey. 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 real-time PCR and microarray analysis.
For isolation of high quality RNA from animal tissue, plant tissue or cultured cells, we offer two approaches: 1) Column-free ready to use TRIsure™ or 2) ISOLATE spin column Kits.

TRIsure™

A ready-to-use solution for the isolation of total RNA

PRODUCT	PACK SIZE	CAT NO.
TRIsure	100ml	BIO-38032
TRIsure	200ml	BIO-38033
TRIsure Plus Bacterial RNA Isolation Kit	100 Preps	BIO-38038
TRIsure Plus Bacterial RNA Isolation Kit	200 Preps	BIO-38039
Bacterial Enhancement Reagent	20ml	BIO-38037

FEATURES

- **Quick isolation of high-quality RNA**
- **Perfect for a wide variety cells, tissues and bacteria**
- **Simple, cost-effective**
- **Convenient 1hour protocol**
- **Isolated RNA is ready for downstream applications**

APPLICATIONS

Isolation of RNA from:

- **Animal tissues**
- **Cultured cells**
- **Bacteria**
- **Plant tissues**

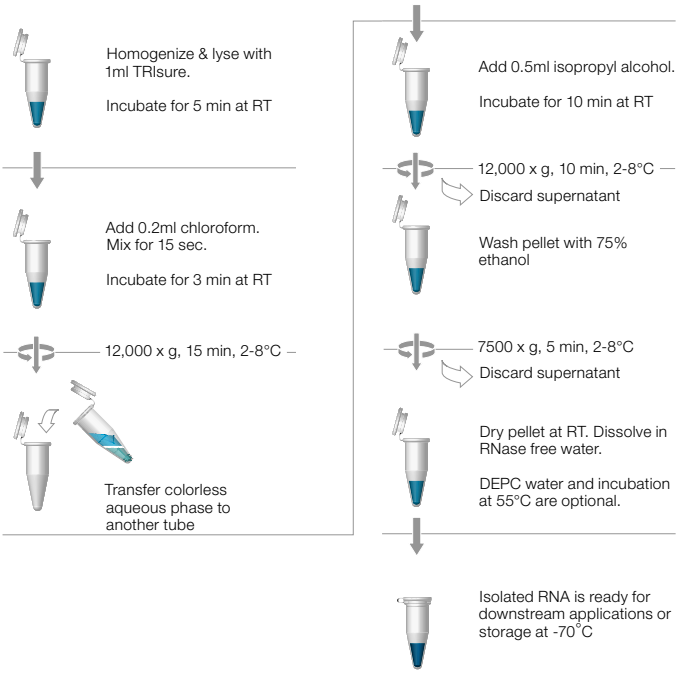
TRIsure is a ready-to-use reagent for the isolation of high quality total RNA from diverse biological materials, including animal tissues, bacteria and cells, as well as plant tissues rich in polysaccharides and proteoglycans.

TRIsure maintains the integrity of the extracted RNA, while disrupting cells and subsequently dissolving cell components. The isolation method is rapid and straightforward (see protocol). High yields of high quality RNA with minimal genomic DNA contamination, is extracted from various samples (Table. 1).

To enhance the isolation of RNA from hard-to-lyse Gram-positive and Gram negative bacterial cells, Bioline has developed **TRIsure Plus Bacterial RNA Isolation Kit**. The kit contains a proprietary Bacterial Enhancement Reagent, in addition to TRIsure, greatly improving the isolation of RNA by promoting protein degradation and inactivating endogenous RNases. The bacterial cells are initially pre-treated with the **Bacterial Enhancement Reagent** and incubated at high temperature (4 minutes at 95˚C). TRIsure is then added to dissolve the cell components and maintain the integrity of the RNA. Higher yields (several fold increases) are obtained due to efficient lysis of bacterial cells (Table. 2), eliminating the need for time consuming mechanical or enzymatic lysis steps.

Using intact RNA as provided by **TRIsure** and **TRIsure Plus Bacterial RNA Isolation Kit**, is a key element in obtaining reliable gene expression data in downstream applications such as RT-PCR, microarray, hybridization assays, and *in vitro* translation.

TRIsure Protocol



Note: TRIsure is a trademark of Bioline.

Protocol

Reagents Required (not supplied):

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

Note: 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.
Gram positive and Gram negative bacteria: Transfer up to 1.5ml (10^8 log phase cells) to a pre-chilled microfuge tube. Pellets cells at 6,000xg for 5 minutes at 2-8˚C. Preheat 200µl Bacterial Enhancement Reagent to 95˚C. Add to cell pellet and mix by pipetting. Incubate at 95˚C for 4 minutes. Add 1ml TRIsure and mix well.

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

Phase Separation: Incubate samples for 5 minutes at room temperature. Add 0.2 ml of chloroform per 1 ml of TRIsure. Cap tubes securely and shake vigorously by hand for 15 seconds. Centrifuge at 12,000 x g for 15 minutes at 2-8°C.
RNA Precipitation: Transfer the colorless upper aqueous phase very carefully, without disturbing the interphase, to another tube. Precipitate the RNA by mixing with 0.5 ml of cold isopropyl alcohol per 1 ml of TRIsure used. Incubate samples for 10 minutes at room temperature then centrifuge at 12,000 x g for 10 minutes at 2-8°C.
RNA Wash: Remove the supernatant. Wash the pellet once with at least 1 ml of 75% ethanol per 1 ml of TRIsure used. Vortex samples and centrifuge at 7,500 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 -20°C.
Re-dissolving the RNA: Air-dry the pellet and dissolve in RNase free water (BIO-37080) or DEPC treated water (BIO-38030) by pipetting the solution up and down. Incubate for 10 minutes at 55-60°C if necessary. Store RNA between -20°C and -70°C.

Note: RNA Precipitation.
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.

Table 1. Expected yield of RNA from different samples using TRIsure

Sample type	Sample quantity	Expected yield
Cultured epithelial cells	1 x 10^6	8-15µg
Cultured fibroblasts	1 x 10^6	20-25µg
Mouse kidney tissue	1mg	2-5µg
Mouse liver tissue	1mg	5-10µg

Table 2. Expected yield of RNA from different bacteria using TRIsure Plus Bacterial RNA Isolation Kit

Sample type	Sample quantity	Expected yield
Gram-negative bacteria (e.g. *E. coli*)	1 x 10^8	>30µg
Gram-positive bacteria (e.g. *Lactococcus lactis*)	1 x 10^8	~30µg

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.*

ISOLATE Plant RNA Mini Kit

Perfect for isolation of pure RNA from plant tissues

PRODUCT	PACK SIZE	CAT NO.
ISOLATE Plant RNA Mini Kit	10 Preps	BIO-52039
ISOLATE Plant RNA Mini Kit	50 Preps	BIO-52040
ISOLATE Plant RNA Mini Kit	250 Preps	BIO-52041

FEATURES

- **Rapid protocol: 30 minutes after homogenization**
- **High purity RNA**
- **Complete removal of genomic DNA**
- **Isolated RNA is ready for downstream applications**

APPLICATIONS

Isolation of RNA from:

- **Fresh plant tissue**
- **Frozen plant tissue**

ISOLATE Plant RNA Mini Kit is specially designed for the fast and efficient isolation of extremely pure total RNA from a variety of plant tissues, including leaves, bark, roots, fruits, etc. Up to 100mg starting material can be processed per spin column (see protocol). The isolated RNA shows excellent performance in downstream applications such as RT- PCR (Fig. 1), reverse transcription (Fig. 2), Northern blot analysis, microarrays and RNA protection assays.

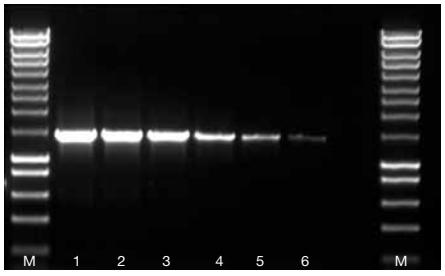


Fig. 1 High yield cDNA obtained from isolated RNA.
RNA was isolated from 20mg freeze-dried budding leaves of *Arabidopsis thaliana* using ISOLATE Plant RNA Mini Kit. cDNA was synthesized using cDNA Synthesis Kit and diluted serially. PCR was performed using MangoMix (BIO-25033) to amplify a 1.4Kb fragment of the allene oxide synthase gene. Products were run on a 1.5% agarose gel. Lanes: HyperLadder I (M), 1µl cDNA (1), 2-fold dilution (2), 4-fold dilution(3), 8-fold dilution (4), 16-fold dilution (5), 32-fold dilution (6).

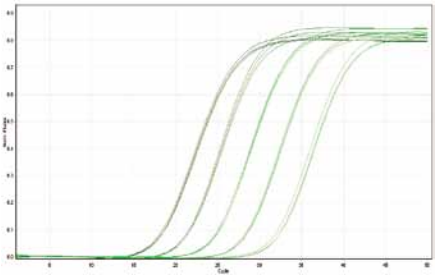
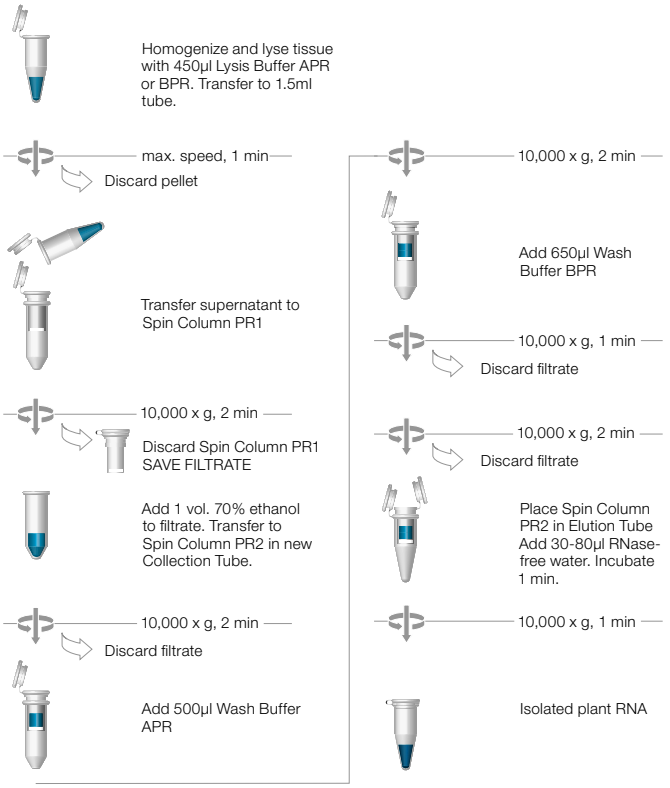


Fig. 2 Superior performance in real-time applications.
RNA was isolated from 20mg freeze-dried budding leaves of *Arabidopsis thaliana* using ISOLATE Plant RNA Mini Kit. cDNA was synthesized using cDNA Synthesis Kit (BIO-65026) and diluted serially 10-fold. Real-time PCR was performed with SensiMix SYBR Kit (QT605-02) using primers designed against the UBQ10 gene.

Total RNA isolation from plant tissue Protocol



ISOLATE RNA Mini Kit

Fast and efficient spin-column isolation of pure RNA

PRODUCT	PACK SIZE	CAT NO.
ISOLATE RNA Mini Kit	10 Preps	BIO-52042
ISOLATE RNA Mini Kit	50 Preps	BIO-52043
ISOLATE RNA Mini Kit	250 Preps	BIO-52044

FEATURES

- **Rapid protocol: 15-20 minutes**
- **High purity RNA**
- **Complete removal of genomic DNA**
- **Isolated RNA is ready for downstream applications**

APPLICATIONS

Isolation of RNA from:

- **Animal tissues**
- **Cultured cells**
- **Bacteria**
- **Plant tissues**

ISOLATE RNA Mini Kit is specially designed for the fast and efficient isolation of extremely pure total RNA from a variety of samples. The kit is compatible with animal tissues, cultured cells and bacterial cells (see protocol and Fig. 1). The isolated RNA shows excellent performance in downstream applications such as reverse transcription, real-time PCR (Fig. 2), Northern blot analysis, microarrays and RNA protection assays.

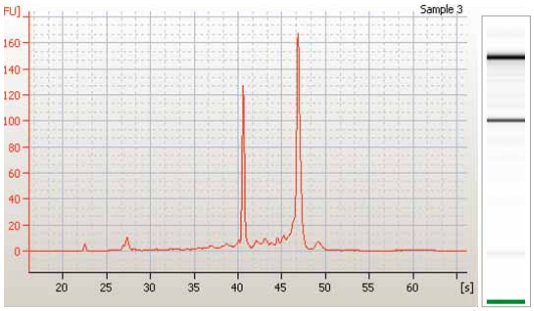


Fig. 1 High quality RNA.
RNA was isolated from HeLa cells using ISOLATE RNA Mini Kit and analyzed using the Bioanalyzer 2100 (Agilent Technologies). The quality of RNA was found to be exceptional (RIN: 9.2).

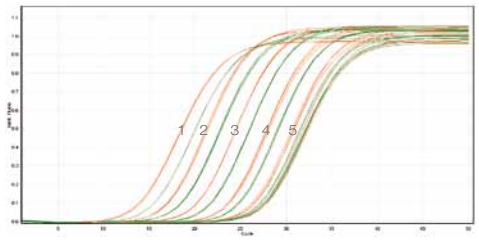
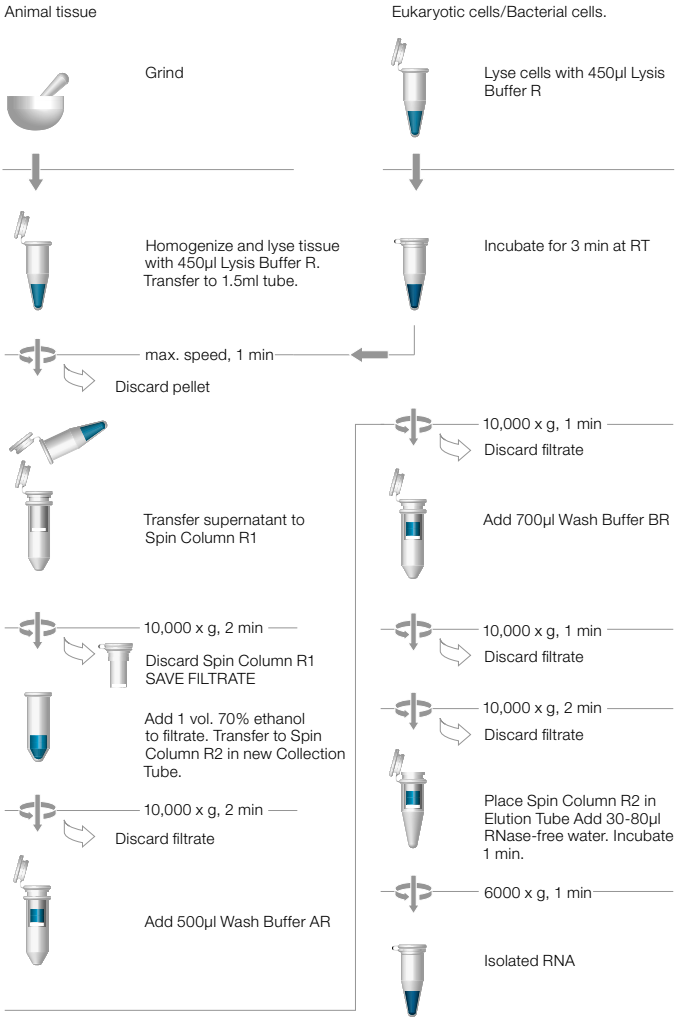


Fig. 2 Superior performance in real time PCR applications.
RNA was isolated from mouse 3T3 cells diluted 10-fold using ISOLATE RNA Mini Kit and Competitor Q's kit. Subsequently, real-time reverse transcriptase reactions were performed using SensiMix SYBR One-Step Kit (QT245-02).
Red traces: RNA isolated using ISOLATE RNA Mini Kit.
Green traces: RNA isolated using Competitor Q's kit
1: 14000 cells, 2: 1400 cells, 3: 140 cells, 4: 14 cells, 5: 1.4 cells

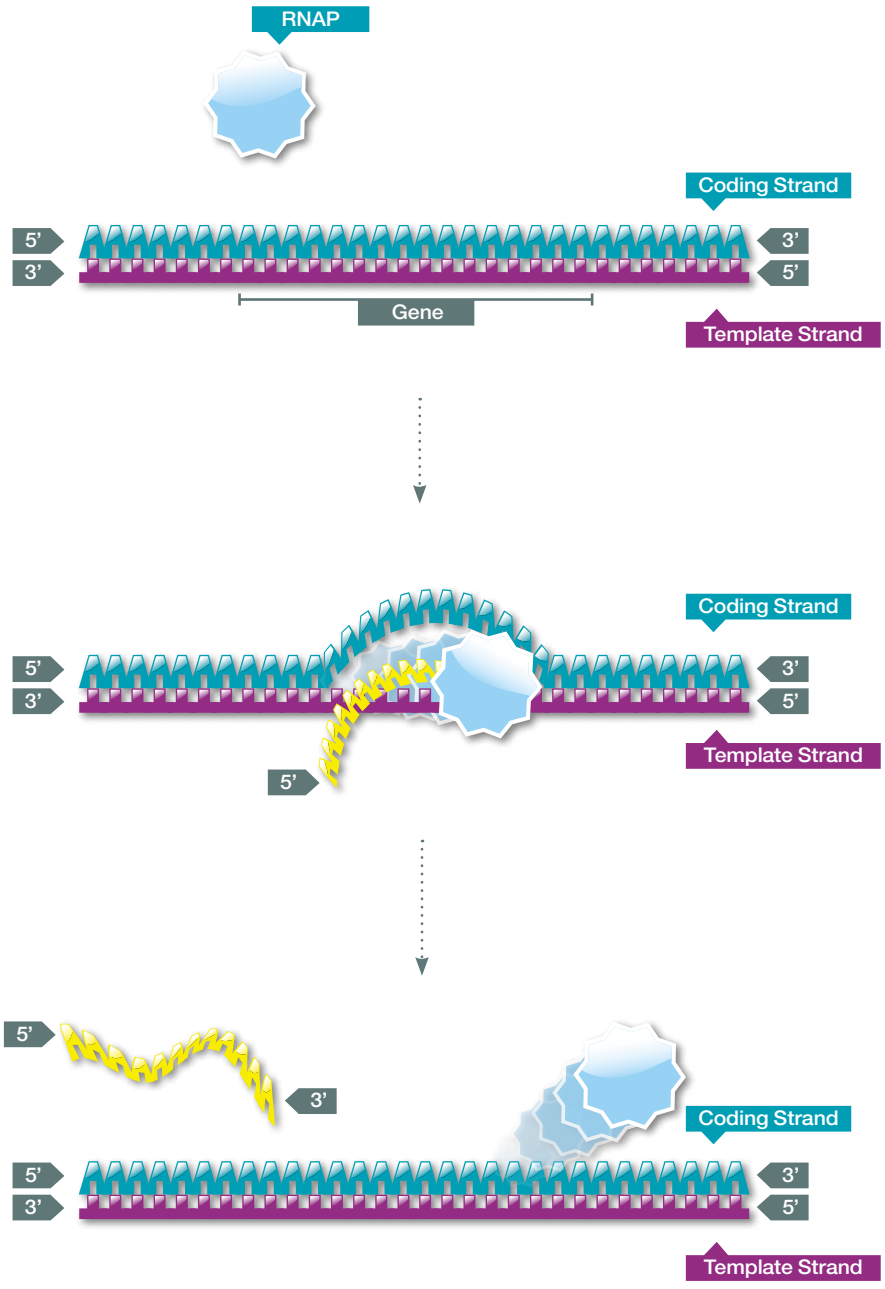
Total RNA isolation Protocol



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 Transcription Kit

Exceptional performance and reliability in transcription

PRODUCT	PACK SIZE	CAT NO.
T7 Transcription Kit	50 Reactions	BIO-21072

FEATURES

- Fast and efficient RNA transcription from any DNA template containing a T7 promotor
- High yield production of short and long RNA
- Reliable and cost-effective
- Optimized reagents for T7 transcription reactions

APPLICATIONS

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

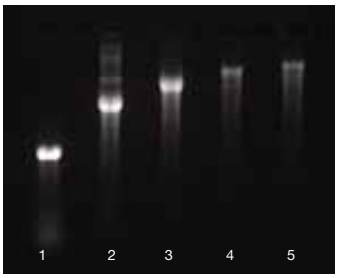


Fig. 1 PCR products of different lengths *in vitro* transcribed using the T7 Transcription Kit.
Lanes: 1Kb RNA product (1), 3Kb RNA product (2), 5Kb RNA product (3), 7Kb RNA product (4), 9Kb RNA product (5)

The T7 Transcription Kit contains all the necessary components to carry out fast and efficient transcription of RNA from any DNA with a T7 promotor sequence (Fig. 1). It can also be used to produce RNA from cloned inserts. The T7 RNA polymerase will incorporate labeled NTPs, and products of this enzyme can be used as probes for screening purposes. The kit contains Bioline RiboSafe RNase inhibitor and Ultimate Reaction Buffer, a proprietary buffer specially formulated and optimized to give exceptional performance and reliability.

The T7 Transcription Kit contains enough reagents for either 50 x 20µl or 1 x 1ml reactions of high yield RNA transcription.

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
T7 RNA 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 being transcribed. 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

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

FEATURES

- 98% pure by HPLC
- Validated for *in vitro* transcription
- Choice of ready-to-use mix or set pack
- DNase, RNase and Nickase free

APPLICATIONS

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

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).

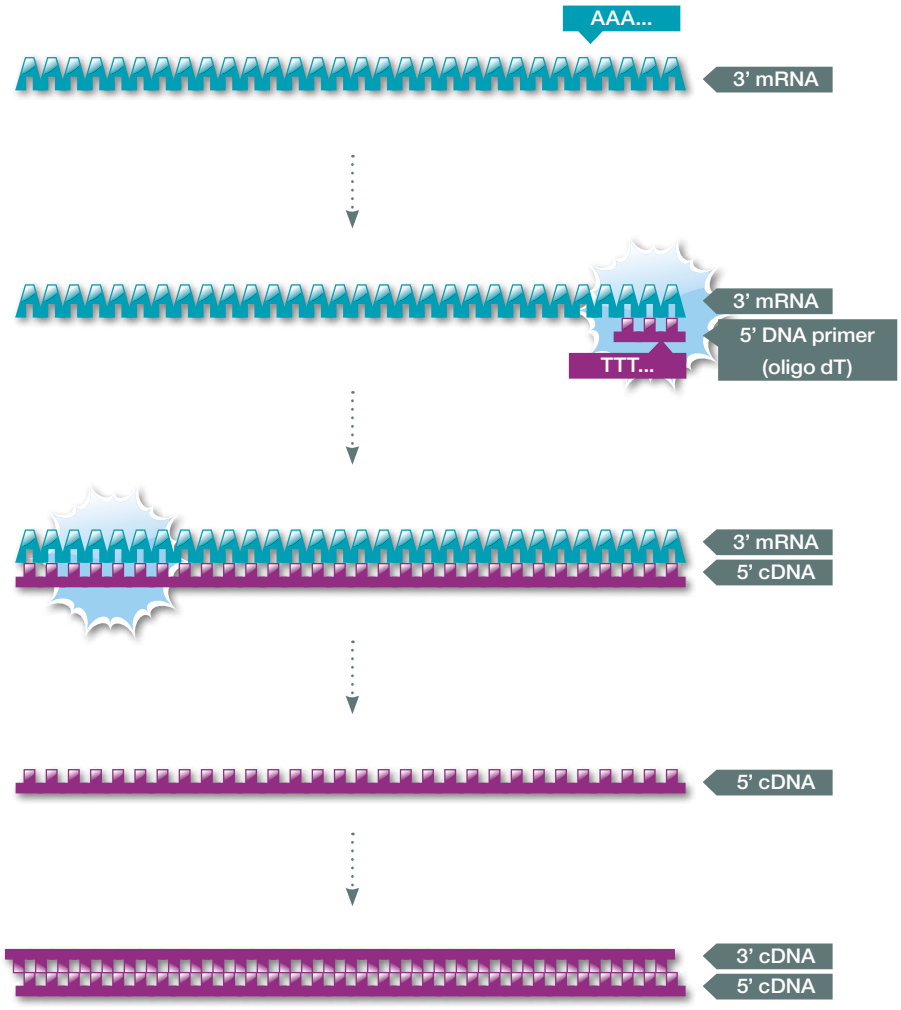
The NTP Set consists of 4 separate 100mM solutions: ATP, GTP, CTP, and UTP (pH 7.5) as sodium salts. Each solution contains 25µmol (250µl) of the corresponding NTP. For *in vitro* RNA synthesis, mix equal volumes of all separate NTP solutions.

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™

Ultra-stable MMLV Reverse Transcriptase

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

FEATURES

- Unrivalled stability
- Working temperature range 37°- 50°C
- Highly sensitive for enhanced cDNA yield
- Produces high quality cDNA ideal for real-time PCR
- Reverse transcribes RNA templates up to 9Kb

APPLICATIONS

- First strand cDNA synthesis for real-time PCR
- cDNA library construction
- mRNA 5' end mapping by primer extension
- Dideoxynucleotide sequencing
- End-labelling of DNA

BioScript™ is a Moloney Murine Leukaemia Virus (MMLV) Reverse Transcriptase, which exhibits high stability, with no loss of activity following 1 week at room temperature. 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, with highly efficient and sensitive transcription, from as little as 100pg, up to 2mg of RNA (Fig. 1).

Many RNA transcripts form stable secondary structures at lower temperatures, making them less suitable as templates for RT-PCR at those temperatures. BioScript is not only active at 37°C-42°C but also at higher temperatures without a loss of activity, unlike many of the competitors (Fig. 2).

BioScript is suitable for first-strand cDNA synthesis, with total RNA, mRNA and *in-vitro* transcribed RNA and shows excellent performance with gene-specific primers, Oligo (dT) as well as random hexamers, making it perfect for cDNA library construction and the production of templates for RT-PCR analysis of gene expression.

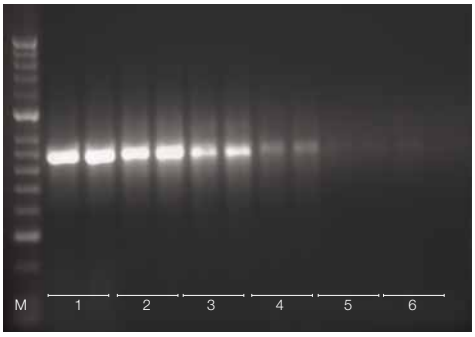


Fig. 1a Reverse-transcription from Mouse Total RNA.
A ten-fold serial dilution of Elite Mouse NIH3T3 Total RNA (1µg to 100pg) was reverse transcribed using 50 Units of BioScript and oligo dT(18) in a 20µl reaction volume. The resultant cDNA was then used as template in a PCR using primers for amplification of a 700bp fragment from mouse b-actin. PCR was performed using ImmoMix in a 50µl reaction. Lanes 1-5 correspond to PCR product from the serial dilution above, reactions were carried out in duplicate. Hyperladder II (M).

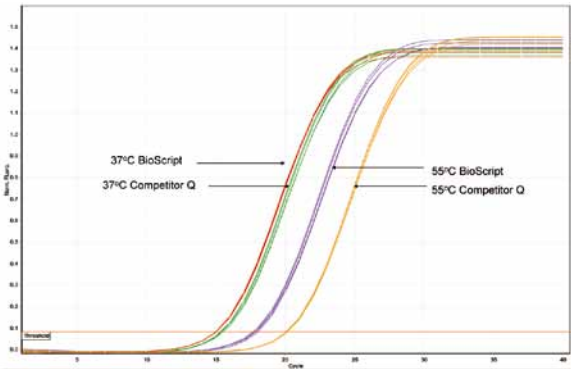


Fig. 2 Amplification of γ-actin at 37°C and 55°C.
γ-actin (left panel) was amplified from cDNA reverse transcribed with BioScript and competitor Q's MMLV at 37°C and 55°C respectively, using SensiMix SYBR (QT-605). While the Ct values for cDNAs amplified at 37°C are relatively close together, the Ct values for 55°C are significantly lower for cDNA synthesized using BioScript, indicating higher thermostability of BioScript.

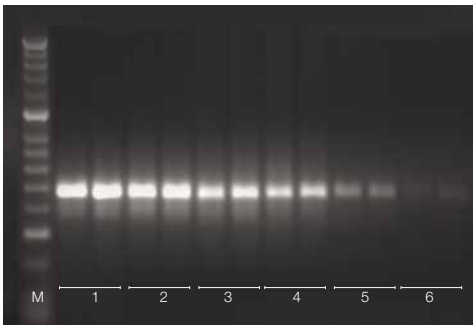


Fig. 1b High sensitivity of BioScript reverse transcriptase.
A ten-fold serial dilution of Elite Human HeLa Total RNA (1µg to 10pg) was reverse transcribed using 50 Units of BioScript and oligo dT(18) in a 20µl reaction volume. The resultant cDNA was then used as a template in a PCR using primers for amplification of a 470bp fragment from human GAPDH. PCR was performed using ImmoMix in a 50µl reaction. Lanes 1-5 correspond to PCR product from the serial dilution above, reactions were carried out in duplicate. Hyperladder II (M).

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

cDNA Synthesis Kit

High quality cDNA from RNA templates

PRODUCT	PACK SIZE	CAT NO.
cDNA Synthesis Kit	30 Reactions	BIO-65025
cDNA Synthesis Kit	100 Reactions	BIO-65026

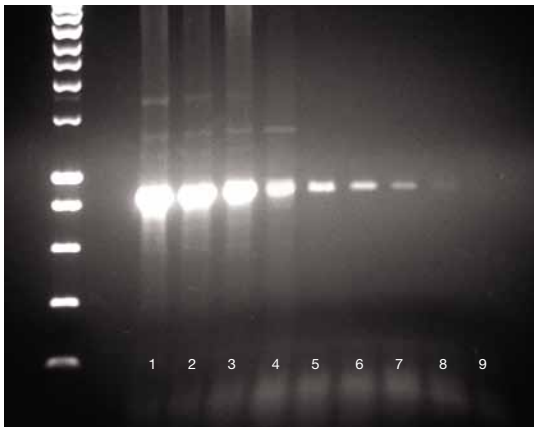
FEATURES

- Generate high quality cDNA for any downstream application
- Highly suited to low concentrations of total RNA down to 100pg
- Convenient, reliable, cost-effective
- Reverse transcribes RNA template up to 9Kb
- Contains RiboSafe RNase Inhibitor to protect RNA template from degradation.

APPLICATIONS

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

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 amplification (Fig. 1). 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 the recombinant Bioline RiboSafe, $(dT)_{18}$ and random hexamer primers together with control RNA template.



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.

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:
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.

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

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

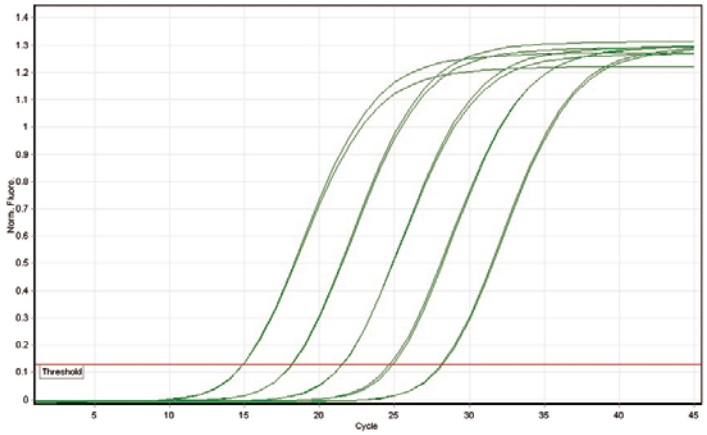


Fig. 1 Two-Step Real-Time PCR using the cDNA Synthesis Kit.
RNA was extracted from mouse kidney tissue samples and cDNA generated using the cDNA Synthesis Kit (a 3:1 ratio of random hexamers to oligo dT). Mouse PGK gene was then amplified from a 10-fold dilutions series of the cDNA over 5 orders of magnitude using the SensiMix SYBR Kit (QT605), to give an r^2 value of 0.999 and a reaction efficiency of 1.

BioScript™ One-Step RT-PCR Kit

Perfect for Gene Expression

PRODUCT	PACK SIZE	CAT NO.
BioScript One-Step RT-PCR Kit	10 Reactions	BIO-65033
BioScript One-Step RT-PCR Kit	25 Reactions	BIO-65030
BioScript One-Step RT-PCR Kit	100 Reactions	BIO-65031

FEATURES

- **Simple to use, one-step set-up**
- **RT-PCR Mix (2x) optimized for as little as 100pg Total RNA**
- **Contains a highly sensitive blend of BioScript and IMMOLASE™**
- **Supplied with highly sensitive reaction buffer containing dNTPs**
- **Highly optimized components for outstanding results**

APPLICATIONS

- **Real-time PCR**
- **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 a unique enzyme formulation, which includes IMMOLASE (our widely used hot-start DNA polymerase) and BioScript, our reverse transcriptase. BioScript possesses low RNase H activity and is highly sensitive even when the amount of template is a limiting factor (Fig. 1).

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 RiboSafe RNase Inhibitor to protect the RNA template from degradation. The proprietary buffer is 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 (Fig. 2).

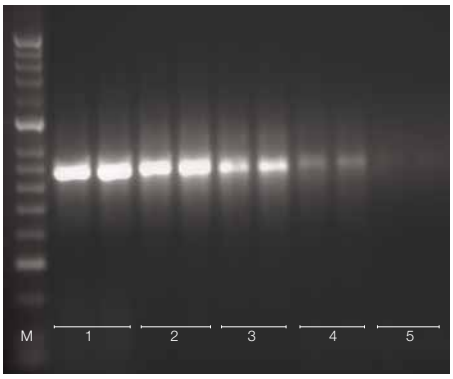


Fig. 1 Reverse-transcription from Mouse Total RNA.
A ten-fold serial dilution of Elite Mouse NIH3T3 Total RNA (1µg to 100pg) was reverse transcribed using 50 Units of BioScript and oligo $dT_{(18)}$ in a 20µl reaction volume. The resultant cDNA was then used as template in a PCR using primers for amplification of a 700bp fragment from mouse β-actin. PCR was performed using ImmoMix in a 50µl reaction. Lanes 1-5 correspond to PCR product from the serial dilution above, reactions were carried out in duplicate. Hyperladder II (M).

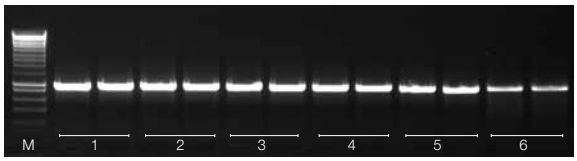


Fig. 2 Amplification of a 1.2kb fragment from Mouse Total RNA.
RT-PCR was carried out on a ten-fold serial dilution of Elite Mouse NIH3T3 Total RNA (100ng to 10pg) using gene-specific primers to the ribosomal RN18S gene. Reverse transcription was performed at 45°C followed by 40 cycles of PCR to amplify a 1.2kb fragment from RN18S. Lanes 1-5 correspond to PCR product from the serial dilution above, reactions were carried out in duplicate. Hyperladder II (M).

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:
Run one cycle at 42°C for 30 minutes, followed by 95°C for 10 minutes (to denature RT enzyme and activate DNA Polymerase), followed by 40 cycles of 95°C for 30 seconds, 50-60°C for 30 seconds and 72°C for 15-30 seconds per kilobase.

3. Mix reactions gently, load into thermocycler and start reaction.

4. Use in gene expression analysis, gene cloning, virus detection, single-cell RT-PCR and other downstream activities.

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.

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 Add 5% DMSO(final reaction concentration)
	Primer dimers	Redesign primers to prevent self-annealing
	Genomic DNA contamination	Try a different target of tissue

Two-step RT real-time PCR

Combining reverse transcription with real-time PCR provides a powerful means to quantify mRNA in cells or tissues. This can be achieved by generating cDNA from Total RNA with the cDNA Synthesis Kit and subsequently performing real-time PCR reaction with the appropriate SensiMix™ Kit.

STEP 1

Step One cDNA Synthesis Kit

The cDNA Synthesis Kit is optimized for RT reactions over a wide range of total RNA concentrations (100pg-2µg), such that long and low-abundance cDNAs can be detected by amplification after cDNA synthesis. The kit contains reverse transcriptase, oligo $(dT)_{18}$ and random hexamer primers together with control RNA template (see page 16). The cDNA generated is used as a template for the appropriate SensiMix product in accordance with the user requirements (for a complete list of instrument compatibility please refer to www.bioline.com/sensimixgraph).

STEP 2

SensiMix SYBR Kit

SensiMix SYBR contains all the components necessary to perform real-time PCR assays with the cDNA templates. The kits deliver highly specific, reproducible and sensitive results across the widest possible dynamic range of template concentrations

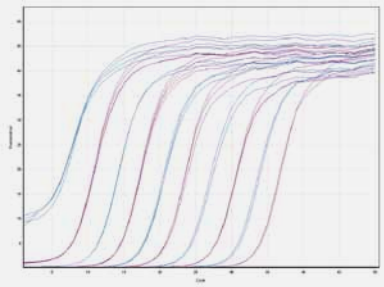
SensiMix SYBR Low-ROX Kit

SensiMix SYBR Low-ROX is the same high performance product as SensiMix SYBR, designed for superior sensitivity and specificity and giving the option of using ROX as a passive reference signal for normalization of the data, particularly for block-based platforms which are more sensitive to the dye levels

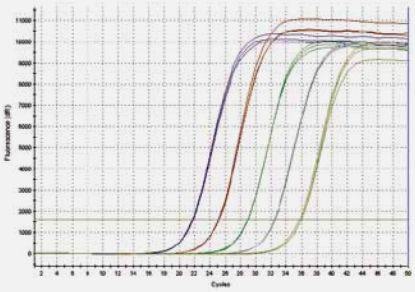
SensiMix SYBR No-ROX Kit

SensiMix SYBR No-ROX is a high-performance product designed for superior sensitivity and specificity on various real-time instruments, in which a passive reference signal is not required

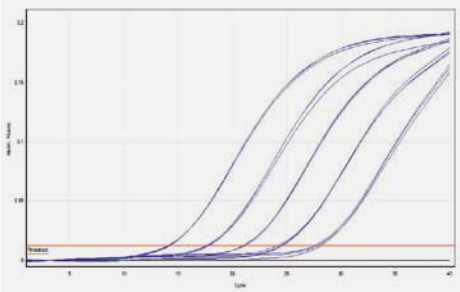
RESULTS



Broad dynamic range using the SensiMix SYBR Kit.
Highly reproducible and efficient real-time PCR of a fragment of the human GAPDH gene was achieved across the 10-fold dilution series of cDNA created using the cDNA synthesis kit. Amplification plots were generated using 10-fold dilutions of cDNA in quadruplicate.



Broad dynamic range achieved using the SensiMix SYBR Low-ROX Kit.
A fragment of the mouse γ-actin gene was amplified from a 10-fold dilution series of NIH3T3 cDNA over 5 orders of magnitude. The use of ROX passive reference dye gives minimal variability.

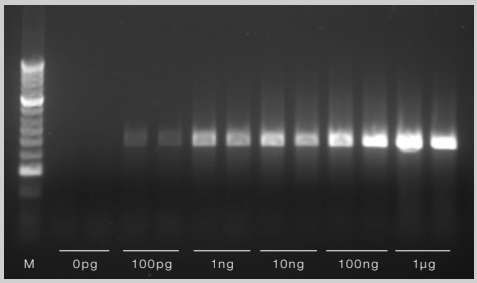


Excellent sensitivity and efficiency using the SensiMix SYBR No-ROX Kit.
High sensitivity and efficient real-time PCR of a fragment of the human GAPDH gene was achieved across a 10-fold dilution series of template. Amplification plots were generated using 10-fold dilutions of a recombinant plasmid tested in triplicate over five orders of magnitude.

Quantify mRNA in cells or tissues

PRODUCT	PACK SIZE	CAT NO.
SensiMix SYBR Kit	250 x 50µl	QT605-02
SensiMix SYBR Kit	500 x 50µl	QT605-05
SensiMix SYBR Kit	2000 x 50µl	QT605-20
SensiMix SYBR Low-ROX Kit	250 x 50µl	QT625-02
SensiMix SYBR Low-ROX Kit	500 x 50µl	QT625-05
SensiMix SYBR Low-ROX Kit	2000 x 50µl	QT625-20

PRODUCT	PACK SIZE	CAT NO.
SensiMix SYBR No-ROX Kit	250 x 50µl	QT650-02
SensiMix SYBR No-ROX Kit	500 x 50µl	QT650-05
SensiMix SYBR No-ROX Kit	2000 x 50µl	QT650-20
SensiMix II Probe Kit	250 x 50µl	BIO-91002
SensiMix II Probe Kit	500 x 50µl	BIO-91005
SensiMix II Probe Kit	2000 x 50µl	BIO-91020
SensiMix SYBR & Fluorescein Kit	250 x 50µl	QT615-02
SensiMix SYBR & Fluorescein Kit	500 x 50µl	QT615-05
SensiMix SYBR & Fluorescein Kit	2000 x 50µl	QT615-20



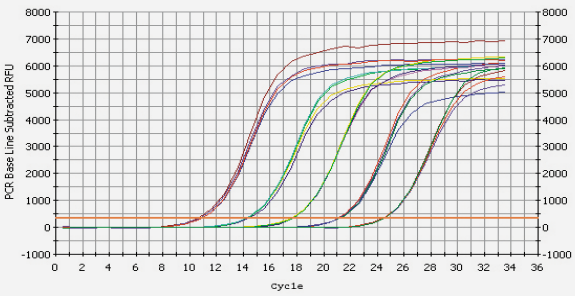
Sensitivity of cDNA Synthesis Kit .
RT-PCR was carried out on HeLa cell total RNA, using a 10 fold dilution series from 1µg down to 100pg with GAPDH gene-specific primers. Reverse transcription was performed at 45°C for 30 mins followed by PCR, to produce a 470bp amplicon. HyperLadder II (M).

SensiMix SYBR & Fluorescein Kit

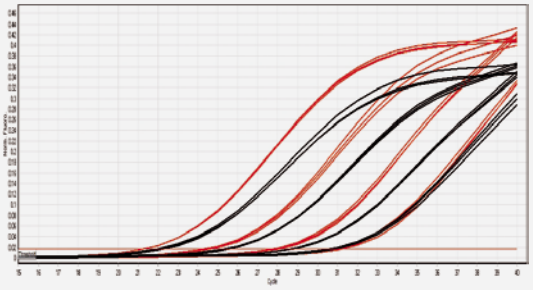
SensiMix SYBR & Fluorescein Kit contains fluorescein, making it specifically suitable for use on real-time instruments that measure fluorescein passive signal, particularly block-based instruments where fluorescein is required to allow calculation of dynamic well factors, allowing as few as 10 copies of a target gene can be detected

SensiMix II Probe Kit

SensiMix II Probe is a ready-to-use mastermix, for highly sensitive and accurate real-time PCR using sequence-specific probe chemistries. It uses the latest advances in technology to deliver superior performance in genotyping, gene expression analysis, and multiplexing



Broad dynamic range and reduced primer dimer using the SensiMix SYBR & Fluorescein Kit.
The mouse YWHAZ gene was amplified from a 10-fold dilution series of cDNA, in quadruplicate, over 5 orders of magnitude, the fluorescein included in the master mix allows for the collection of dynamic well factors, removing inconsistencies due to pipetting from the results.



Comparison of SensiMix II Probe (red) with SensiMix Probe (black).
Human γ-actin gene was amplified in triplicate using a conventional primer/probe set with a FAM labeled probe from 10-fold serial dilution of cDNA with either SensiMix II Probe (red), or SensiMix Probe (black). SensiMix II Probe showed earlier Cts (1-2 Cts) for both genes and higher signal and greater sensitivity.

CellSure cDNA Kit

cDNA directly from cultured cells for analysis by PCR

PRODUCT	PACK SIZE	CAT NO.
CellSure cDNA Kit	30 Reactions	BIO-65040
CellSure cDNA Kit	100 Reactions	BIO-65041

FEATURES

- From cells to cDNA in 90 minutes
- No RNA extraction required
- Quick and easy production of cDNA directly from cell culture
- DNase removal of genomic DNA for detection of RNA only

APPLICATIONS

- The cDNA is suitable for standard PCR assay
- Real-time PCR assays in combination with SensiMix Kits
- 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 (Fig. 1). 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 (Fig. 2).

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 (Fig. 3).

CellSure cDNA Kit contains BioScript, Bioline´s reverse transcriptase, which is active over a wide range of temperatures.

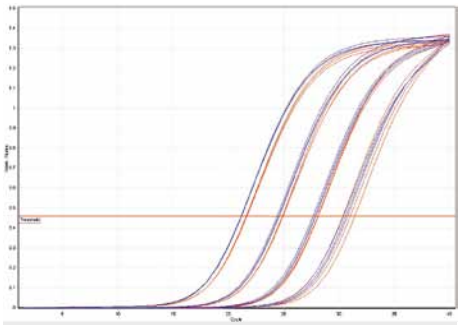


Fig. 1 CellSure cDNA Kit in two-step RT-PCR using SYBR Green I chemistry.
DNA synthesized from cell lysates containing approximately 625 to 0.625 cells were prepared using CellSure cDNA Kit and Competitor A's kit. The cDNA was subsequently used in real-time PCR reactions using SensiMix and SYBR Green 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 and Competitor A's kit respectively.

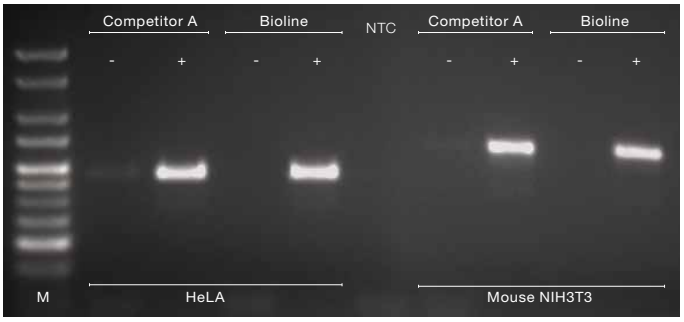


Fig. 2 Amplification of a fragment of the β-actin gene from cDNA obtained from HeLa and NIH3T3 cells using Bioline's and Competitor A's kits.
Approximately 2.5 x 10^3 cells were used in a 25µl PCR reaction using BIOTAQ DNA Polymerase. HyperLadder V (M).

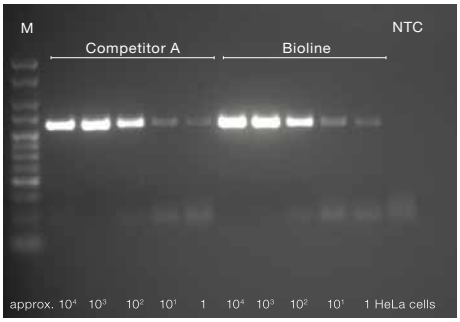


Fig. 3 Amplification of a fragment of the GAPDH gene from cDNA obtained from HeLa cells using the CellSure cDNA Kit and Competitor A's kit.
A serial dilution of the lysate was performed prior to RNase inactivation, DNase treatment and cDNA synthesis. The approximate number of cells used per reaction is as indicated in the figure. HyperLadder V (M).

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

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 1-2,000 cells per µl of lysis buffer. To ensure optimum lysis conditions, do not lyse in more than 100µl, but do not exceed 2,000 cells per µl as this may inhibit the RT-PCR reaction.

For a small number of cells (≤10,000 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 -80ºC for up to 2 months. Lysates made from ≤2.5 x 10^4 cells should be used for RT-PCR immediately.

Generation of cDNA:
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.

Two-step RT-PCR:
For high quality RT-PCR on the cDNA we recommend using VELOCITY DNA Polymerase as followed:

1. In 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 CellSure cDNA kit
10 x PCR buffer	5µl	BIO-21098
50mM $MgCl_2$	1.5µl	
10mM dNTP mix	4µl	Supplied with CellSure cDNA Kit
Forward primer	200-900 nM	*Control supplied with CellSure cDNA Kit
Reverse primer	200-900 nM	
VELOCITY DNA Polymerase	2 units	BIO-21098
Nuclease-free dH_2O	Up to 50µl	BIO-37080

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

2. Run one cycle at 95°C for 2 minutes, followed by 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and a final cycle of 72°C for 5 minutes.

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.

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

Oligo $(dT)_{18}$

Primer for first strand cDNA synthesis

PRODUCT	PACK SIZE	CAT NO.
Oligo $(dT)_{18}$	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

PRODUCT	PACK SIZE	CAT NO.
Random Hexamer Primers	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.

RNA Templates and Controls

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

Elite RNA controls

Validated total RNA controls

PRODUCT	PACK SIZE	CAT NO.
Elite Human HEK293 Total RNA	100µg	BIO-38034
Elite Human HeLa Total RNA	100µg	BIO-38035
Elite Mouse NIH3T3 Total RNA	100µg	BIO-38036

FEATURES

- **Ready-to-use**
- **Elite Human HEK293 RNA from embryonic kidney cells, Human HeLa RNA from cervical cells, Elite Mouse NIH3T3 RNA from fibroblast cells**
- **DNase I treated to minimize genomic DNA contamination**

APPLICATIONS

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

The total RNA of the three Elite products is first isolated using TRIsure™ (BIO-38032). Then, the RNA is DNase I treated, precisely quantified and re-suspended 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, Northern analysis, ribonuclease protection assays, S1 nuclease assays and *in vitro* translation.

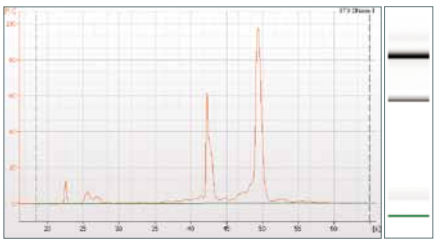


Fig. 1 RNA Quality.
Total RNA extracted from NIH3T3 cells with TRIsure and DNase I treated. Agilent Bioanalyzer show extremely high quality RNA (RIN = 9.9, 28s/18s = 2).

RNA Reagents

Bioline is committed to provide high quality general reagents to facilitate your research with RNA, these products are described below. Once RNA has been isolated, it can be visualized by agarose electrophoresis. It is recommended to load RNA samples with our specially designed RNA loading buffers and run them with the appropriate Crystal buffers. After cDNA synthesis, co-precipitants can be used to ensure maximum recovery of nucleic acids.

Agarose & Agarose Tablets

Perfect for gel electrophoresis of RNA

PRODUCT	PACK SIZE	CAT NO.
Agarose, Molecular Grade	100g	BIO-41026
Agarose, Molecular Grade	500g	BIO-41025
Agarose, HiRes Grade	100g	BIO-41029
Agarose Tablets	100g	BIO-41028
Agarose Tablets	500g	BIO-41027

FEATURES

- DNase/RNase-free
- Excellent value and gel clarity
- High gel strength (>1500g/cm^2)
- Choice of powder or exactly pre-weighed tablets

APPLICATIONS

- Gel electrophoresis of RNA samples

Bioline's Agarose, Molecular Grade is ideally suited for routine analysis of nucleic acids by gel electrophoresis. Bioline's extremely pure molecular biology grade agarose has no detectable DNase or RNase activity and forms strong gels with low background.

Bioline pre-weighed Agarose Tablets 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.

Crystal 50x TAE buffer

Native and denaturating RNA analysis

PRODUCT	PACK SIZE	CAT NO.
Crystal 50x TAE Buffer	5 Pouches	BIO-37103

FEATURES

- Exactly pre-weighed powder in sealed pouches
- Dissolve and use in minutes
- Reagents of the highest purity and quality
- RNase and DNase free

APPLICATIONS

- Native and denaturing RNA analysis
- Agarose gel electrophoresis of long fragments (>1500bp)

Crystal 50x TAE buffer is a Tris-Acetate-EDTA electrophoresis running buffer made from reagents with the highest purity and delivered in convenient ready-to-use sealed pouches, containing a pre-determined amount of components. Simply dissolve the contents in deionized water to the recommended volume and the buffer is ready for use. Bioline Crystal buffers undergo extensive and stringent QC assays and the guaranteed batch-to-batch reproducibility provides consistent results.

One pouch of Crystal 50x TAE buffer dissolved in deionized water and made up to 1000ml yields a 10x stock solution containing 2.0M Tris-Acetate, 0.05M EDTA, pH 8.3 at 25°C.

MOPS-EDTA-Na Acetate Buffer

Developed for agarose gel electrophoresis of RNA

PRODUCT	PACK SIZE	CAT NO.
MOPS-EDTA-Na Acetate Buffer	1 Litre	BIO-38027

FEATURES

- Ready-to-use 10x solution
- DNase/RNase free
- No need for harmful substances such as formamide/formaldehyde

APPLICATIONS

- Agarose gel electrophoresis of RNA samples

Crystal 50x TAE buffer is a Tris-Acetate-EDTA electrophoresis running buffer made from reagents with the highest purity and delivered in convenient ready-to-use sealed pouches, containing a pre-determined amount of components. Simply dissolve the contents of each the pouch in deionized water to the recommended volume and the buffer is ready for use. Bioline Crystal buffers undergo extensive and stringent QC assays and the guaranteed batch-to-batch reproducibility provides consistent results.

One pouch of Crystal 50x TAE buffer dissolved in deionized water and made up to 1000ml yields a 10x stock solution containing 2.0M Tris-Acetate, 0.05M EDTA, pH 8.3 at 25°C.

Crystal RNA Loading Buffers

Specially developed for loading RNA samples

PRODUCT	CONC.	PACK SIZE	CAT NO.
Crystal 2x RNA Loading Buffer with ethidium bromide	2x	1ml	BIO-38025
Crystal 2x RNA Loading Buffer with ethidium bromide	2x	1ml	BIO-38026

FEATURES

- Available with or without ethidium bromide
- Ready-to-use 2x solution
- Guaranteed reproducible results

APPLICATIONS

- Agarose gel electrophoresis of RNA samples
- Northern Blot analysis

Bioline Crystal 2x RNA Loading Buffers maintain the denatured state of the RNA during electrophoresis. The ready-to-use buffer 2x solutions are available either with or without ethidium bromide. If the RNA is to be used in a Northern Blot, we recommend using RNA Loading Buffer without ethidium bromide, since ethidium bromide reduces hybridization efficiency once the RNA is transferred to a membrane.

We recommend using RNA Loading buffer on MOPS and TBE agarose gels. The RNA loading buffer can also be used on formaldehyde, glyoxal, agarose gels and acrylamide-urea gels.

DEPC-treated Water

Ideal for RNA research

PRODUCT	PACK SIZE	CAT NO.
DEPC-treated Water	10 x 10ml	BIO-38030
DEPC-treated Water	1 Litre	BIO-38031

FEATURES

- Ready to use, no mixing or autoclaving needed
- DNase/RNase free
- Ultra-pure 18.2MΩ

APPLICATIONS

- For use in RNA applications

Bioline DEPC-treated Water is deionised, high-quality molecular grade water, which is ready-to-use and requires no preparation, mixing or autoclaving. DEPC-treated Water is prepared by treating ultra-pure 18.2MΩ PCR Water with diethylpyrocarbonate (DEPC), and autoclaving.

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

RiboSafe RNase Inhibitor

Complete inhibition of RNases

PRODUCT	CONC.	PACK SIZE	CAT NO.
RiboSafe RNase Inhibitor	40u/µl	2500 Units	BIO-65027
RiboSafe RNase Inhibitor	40u/µl	10,000 Units	BIO-65028

FEATURES

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

APPLICATIONS

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

Bioline Ribonuclease Inhibitor (RiboSafe RNase Inhibitor) is a recombinant protein which completely inhibits a broad spectrum of eukaryotic RNases, including RNases A, B and C by binding non-covalently in a 1:1 ratio (Fig. 1 & 2). RiboSafe shows no inhibition of polymerase (Fig. 3) or transcriptase activity (Fig. 4) and it is not effective against RNase H, T1, S1-nuclease or RNase from Aspergilus. With an association constant of 1014M, RiboSafe is useful in any applications where the presence of RNases is a potential problem. RiboSafe RNase Inhibitor is tested for activity, SDS-PAGE purity, and the absence of endonucleases, nickases and exonucleases.

The enzyme is supplied at a concentration of 40u/µl.

Typical Reaction Conditions

RiboSafe RNase Inhibitor must be used at a final concentration of between 10-40u in a 25µl reaction mix, this is dependent on the level of RNase contamination in the sample. For optimal RNase inhibition, a final concentration of 1mM DTT is required.

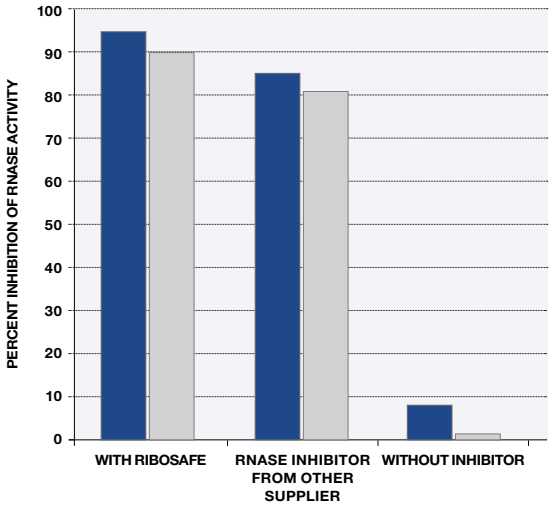


Fig. 1 RiboSafe RNase Inhibitor provides superior RNA protection.
Inhibition of RNase A by RiboSafe RNase Inhibitor and RNase Inhibitor from another supplier was assessed with the total Yeast RNA assay for the measurement of RNase activity (blue columns) and the pre-incubation-assay (grey columns). RiboSafe RNase Inhibitor blocks RNase A with higher efficiency than other commercially available RNase inhibitors.

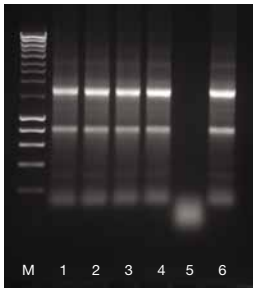


Fig. 2 RiboSafe inhibits increasing amounts of RNase A with high efficiency.
2µg of total human HeLa cell RNA was incubated with 20 Units of RiboSafe RNase Inhibitor and 2ng, 750pg, 250pg and 125pg of RNase A (lanes 1-4) at 37°C for 30 min. Controls were no RiboSafe RNase Inhibitor (lane 5) and total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

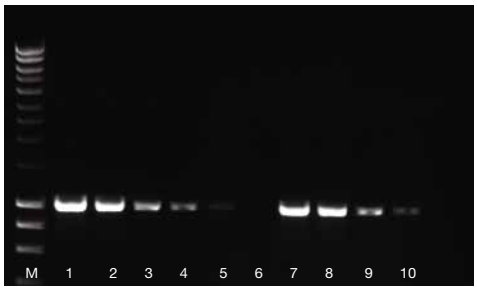


Fig. 3 RiboSafe shows no inhibition of polymerase.
A two-fold serial dilution of Total HeLa cell RNA (1µg - 0.075µg) was reverse transcribed in the presence and in the absence of RiboSafe RNase Inhibitor, followed by the amplification of a 1Kb fragment of the Angiotensin receptor II gene using SAHARA Mix (lanes 1-10). HyperLadder I (M).

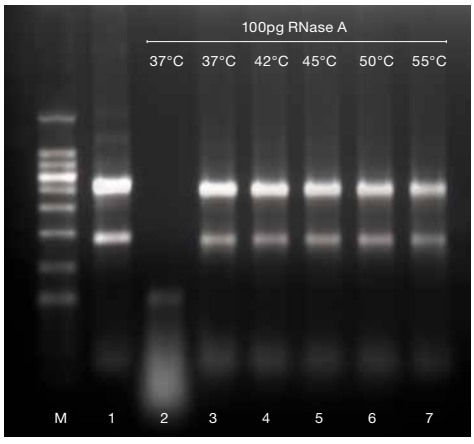
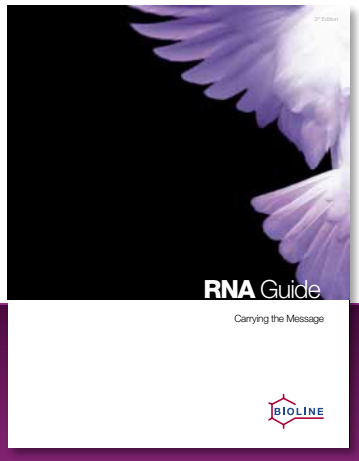


Fig. 4 RiboSafe RNase Inhibitor is active up to temperatures of 55°C.
2µg aliquots of total mouse RNA were incubated with 40 Units of RiboSafe RNase Inhibitor and 100pg of RNase A at various temperatures for 30 minutes. Lanes: RiboLadder Long (M), Total mouse RNA (1), Total mouse RNA with RNase A only (2), Total mouse RNA with RiboSafe RNase Inhibitor and RNase A (3-7).

ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
TRIsure	100ml	BIO-38032
TRIsure	200ml	BIO-38033
TRIsure Plus Bacterial RNA Isolation Kit	100 Preps	BIO-38038
TRIsure Plus Bacterial RNA Isolation Kit	200 Preps	BIO-38039
Bacterial Enhancement Reagent	20ml	BIO-38037
ISOLATE RNA Mini Kit	10 Preps	BIO-52042
ISOLATE RNA Mini Kit	50 Preps	BIO-52043
ISOLATE RNA Mini Kit	250 Preps	BIO-52044
ISOLATE Plant RNA Mini Kit	10 Preps	BIO-52039
ISOLATE Plant RNA Mini Kit	50 Preps	BIO-52040
ISOLATE Plant RNA Mini Kit	250 Preps	BIO-52041
T7 Transcription Kit	50 Reactions	BIO-21072
NTP Set	4 x 25µmol	BIO-39052
NTP Mix	100µmol	BIO-39050
BioScript	10,000 Units	BIO-27036
BioScript	4 x 10,000 Units	BIO-27036-4
cDNA Synthesis Kit	30 Reactions	BIO-65025
cDNA Synthesis Kit	100 Reactions	BIO-65026
BioScript One-Step RT-PCR Kit	10 Reactions	BIO-65033
BioScript One-Step RT-PCR Kit	25 Reactions	BIO-65030
BioScript One-Step RT-PCR Kit	100 Reactions	BIO-65031
SensiMix SYBR Kit	250 x 50µl	QT605-02
SensiMix SYBR Kit	500 x 50µl	QT605-05
SensiMix SYBR Kit	2000 x 50µl	QT605-20
SensiMix SYBR Low-ROX Kit	250 x 50µl	QT625-02
SensiMix SYBR Low-ROX Kit	500 x 50µl	QT625-05
SensiMix SYBR Low-ROX Kit	2000 x 50µl	QT625-20
SensiMix SYBR No-ROX Kit	250 x 50µl	QT650-02
SensiMix SYBR No-ROX Kit	500 x 50µl	QT650-05
SensiMix SYBR No-ROX Kit	2000 x 50µl	QT650-20
SensiMix II Probe Kit	250 x 50µl	BIO-91002
SensiMix II Probe Kit	500 x 50µl	BIO-91005
SensiMix II Probe Kit	2000 x 50µl	BIO-91020
SensiMix SYBR & Fluorescein Kit	250 x 50µl	QT615-02
SensiMix SYBR & Fluorescein Kit	500 x 50µl	QT615-05
SensiMix SYBR & Fluorescein Kit	2000 x 50µl	QT615-20
CellSure cDNA Kit	30 Reactions	BIO-65040
CellSure cDNA Kit	100 Reactions	BIO-65041
Elite Human HEK293 Total RNA	100µg	BIO-38034
Elite Human HeLa Total RNA	100µg	BIO-38035
Elite Mouse NIH3T3 Total RNA	100µg	BIO-38036
Oligo $(dT)_{18}$	27µg	BIO-38029
Random Hexamer Primers	25µg	BIO-38028
Agarose, Molecular Grade	100g	BIO-41026
Agarose, Molecular Grade	500g	BIO-41025
Agarose, HiRes Grade	100g	BIO-41029
Agarose Tablets	100g	BIO-41028
Agarose Tablets	500g	BIO-41027
Crystal 50x TAE Buffer	5 Pouches	BIO-37103
MOPS-EDTA-Na Acetate Buffer	1 Litre	BIO-38027
Crystal 2x RNA Loading Buffer with ethidium bromide	1ml	BIO-38025
Crystal 2x RNA Loading Buffer with ethidium bromide	1ml	BIO-38026
DEPC-treated Water	10 x 10ml	BIO-38030
DEPC-treated Water	1 Litre	BIO-38031
RiboSafe RNase Inhibitor	2500 Units	BIO-65027
RiboSafe RNase Inhibitor	10,000 Units	BIO-65028



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TECHNICAL SUPPORT

For technical assistance or more information on these products, please call us on:

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