



**Product Insert**  
**CellSure cDNA Kit**

**Catalogue Numbers:**

BIO-65040                      30 Reactions  
BIO-65041                      100 Reactions

**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

**Description**

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.

**Product Specifications**

**Batch details:**  
Batch No:                      See vial  
Units per vial:                See vial  
Concentration:                See vial

**Storage Conditions:**  
CellSure cDNA kit contents can be stored for up to 6 months at -20°C except for the Mouse Total RNA, which should be stored at -80 °C.

**Shipping Conditions:**  
On Dry Ice or Blue Ice

**Associated Products:**

Product Name	Pack Size	Cat No
IMMOLASE	250 Units	BIO-21046
RiboSafe RNase Inhibitor	2500 Units	BIO-65027

**Notes**

1. This product insert is a declaration of analysis at the time of manufacture.
2. For research use only.
3. SybrGreen® is a trademark of Molecular Probes

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**CellSure cDNA Kit Components**

Component	BIO-65040 30 Reactions	BIO-65041 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) <sub>18</sub> (50µM)	60µl	200µl	5' TTTTTTTTTTTTTTTTTT 3' for reverse transcription where gene specific primers are designed close to the 3'
DEPC-H <sub>2</sub> O	1.75ml	2 x 1.75ml	DEPC-treated H <sub>2</sub> O 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 a  $2 \times 10^5$  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  $2 \times 10^5$  cells into a microcentrifuge tube. Centrifuge at  $200 \times g$  for 5 minutes in a bench centrifuge to pellet the cells.
3. Remove the growth medium and wash cells at least once with  $500 \mu\text{l}$  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  $100 \mu\text{l}$  ice cold Cell Lysis Buffer.

**Control:** For the control reaction, instead of the cells, add  $1 \mu\text{l}$  of Control Mouse Total RNA to  $100 \mu\text{l}$  of Cell lysis buffer.

*Cell lysate concentrations should be 1-2000 cells per  $\mu\text{l}$  of lysis buffer. To ensure optimum lysis conditions, do not lyse in more than  $100 \mu\text{l}$ , but do not exceed 2000 cells per  $\mu\text{l}$  as this may inhibit the RT-PCR reaction.*

*For a small number of cells ( $\leq 10000$  cells) or analysis of low-expressing genes, lyse the cells in a minimum of  $5 \mu\text{l}$  of lysis buffer. For single cell analysis, lyse in  $5 \mu\text{l}$  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  $75^\circ\text{C}$  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  $0.04 \text{ U}/\mu\text{l}$  and incubate at  $37^\circ\text{C}$  for 15 minutes to degrade genomic DNA.
9. Inactivate the DNase I by heating at  $75^\circ\text{C}$  for 5 minutes.
10. Place on ice and the lysate is ready for RT-PCR (lysate can be stored at  $-20^\circ\text{C}$  at this stage).

### **Storage of the cell lysates:**

Lysates made from  $\geq 2.5 \times 10^4$  cells can be stored at  $-20^\circ\text{C}$  for up to 1 week, or at  $-80^\circ\text{C}$  for up to 2 months. Lysates made from  $\leq 2.5 \times 10^4$  cells should be used for RT-PCR immediately.

### **Two-step RT-PCR**

**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  $20 \mu\text{l}$  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 H <sub>2</sub> O	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.

- Heat at 70 °C for 5 minutes.
- Then add:

Component	Amount	Source
5x RT buffer	4µl	Supplied
RNase Inhibitor	1µl	Supplied
Reverse Transcriptase	0.25µl	Supplied
DEPC-treated H <sub>2</sub> O	Up to 20µl	Supplied

- Incubate at 42 °C for 30-60 minutes.
- Heat at 70 °C for 10 minutes to inactivate the BioScript reverse transcriptase.
- Store the RT reaction at -20 °C or proceed to the amplification step.
- 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
10 x PCR buffer	5µl	Not supplied
50mM MgCl <sub>2</sub>	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 <sub>2</sub> O	Up to 50µl	Not supplied

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

- 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

- Analyse 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 optimise 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 75°C. 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.