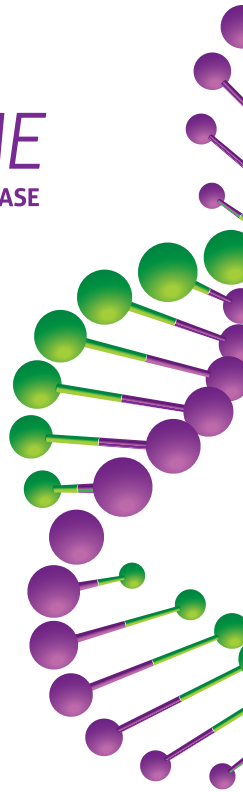
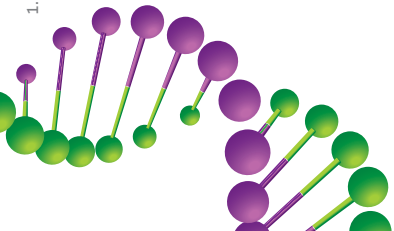
TRANSCRIPTME

REVERSE TRANSCRIPTASE



1.2020



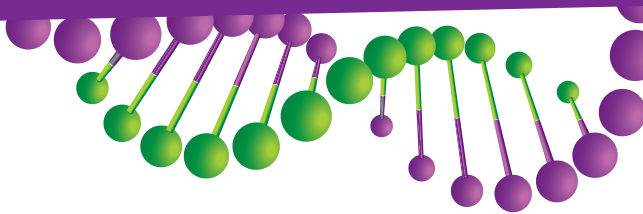
blirt

TRANSCRIPTME

REVERSE TRANSCRIPTASE

TRANSCRIPTME is a modified, recombinant form of the Reverse Transcriptase from Moloney Murine Leukemia Virus (M-MuLV) purified from *Escherichia coli*. ***TRANSCRIPTME*** Reverse Transcriptase synthesizes a complementary DNA strand in the presence of a primer using either RNA (cDNA synthesis) or single-stranded DNA (ssDNA) as a template.

TRANSCRIPTME Reverse Transcriptase has increased thermal stability, that allows the reaction to be carried out at a higher temperature (optimum activity at 50°C). It increases the efficiency and specificity of those transcribed RNA regions which are rich in GC pairs and/or contain secondary structures. The enzyme has no 3'→5' exonuclease and reduced RNase H activity, that improves the synthesis of a full-length cDNA, even from long mRNA templates, using random priming. The enzyme gives high yields of first strand cDNA synthesis up to 10 kb long.



Features and advantages

- → High yields of full-length cDNA synthesis (up to 10 kb long)
- → RNA- and DNA-dependent DNA polymerase activities
- → Increased sensitivity in RT-qPCR and RT-PCR assays
- → Starting material: 10 pg – 5 µg of total RNA or 10 pg – 500 ng of mRNA
- → Optimal reaction temperature: 50°C
- → Increased thermostability
- → No 3'→5' exonuclease activity
- → Reduced RNase H activity
- → Suitable for the amplification of difficult RNA templates

Applications

- → Full-length cDNA template synthesis for RT-qPCR and two-step RT-PCR assays
- → cDNA synthesis for molecular cloning
- → cDNA library construction
- → RNA analysis

TRANSCRIPTME

REVERSE TRANSCRIPTASE

Protocol for the first strand cDNA synthesis

1. Add all reaction reagents listed below to a sterile nuclease-free Eppendorf tube placed **on ice or in a freezing rack** (for a larger quantity of samples, preparing Master Mix without an RNA template is recommended).
   The reagents should be added in the following order:

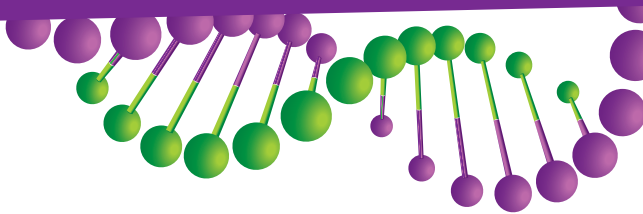
	Reagent	Quantity
RNA	total RNA mRNA	10 pg – 5 µg 10 pg – 500 ng
Primer	oligo(dT)$_{12-18}$ primer mix or random hexamers or specific primers	1 µl (50 µM) 1 µl (50–250 ng) 1 µl (2 pmol)
Nucleotides	10 mM dNTP MIX	1 µl (final conc. 0.5 mM)
Water	nuclease-free water	fill up to 16 µl

Optional denaturation step*: Incubate the sample at 65°C for 5 min, cool on ice, spin briefly and return to the ice.

* If GC-rich templates containing secondary structures are used, then the additional denaturation step is necessary.

2. Add the reagents listed below to the sample in the following order:

Reagent	Quantity
10x RT Reaction Buffer	2 µl
RIBO*PROTECT Hu* RNase Inhibitor (RT-35)	1 µl (40 U)
TRANSCRIPTME Reverse Transcriptase	1 µl (200 U)
Total volume	20 µl



3. Mix gently and spin briefly.

Optional step*: Incubate sample at 25°C for 10 min.
* If random hexamers are used, this step is mandatory.

4. Incubate at 50°C for 30 min.
5. Stop the reaction at 85°C for 5 min and **immediately** cool the sample on ice.
6. The cDNA obtained is ready for direct use in PCR or qPCR (undiluted or diluted in nuclease-free water or TE buffer) or can be stored at -20°C or -70°C.

Working with RNA

Acquisition of high quality, intact RNA, free of genomic DNA and RNase traces, is vital for the synthesis of full-length cDNA followed by an accurate quantitative analysis (qPCR).

The following recommendations for working with RNA should therefore be followed:

- → Maintain aseptic working conditions: use disposable gloves, changing them as frequently, as required; use RNase-free consumables; only work in an area assigned for working with RNA and with equipment designated for that purpose.
- → HL-dsDNase (not included) may be used if obtaining a DNA-free RNA sample is required.
- → RNA samples should be stored aliquoted at -70°C. Avoid subjecting the samples to repeated freezing and thawing cycles.

Additional information

- We recommend ***EXTRACTME*** **TOTAL RNA KIT** (EM09.1) and ***EXTRACTME*** **TOTAL RNA PLUS KIT** (EM11.1) for total RNA isolation from tissues and cell cultures
- During RT-PCR preparation keep ***TRANSCRIPTME*** Reverse Transcriptase and **10x RT** Reaction Buffer on ice or in a freezing rack
- Use an RNase H treatment for reactions sensitive to residue RNA traces in order to increase the sensitivity of RT-qPCR
- The quantity of cDNA used when preparing PCR or qPCR reactions should not exceed 1/10 of a final reaction volume; e.g. a maximum volume of 2.5 µl of cDNA should be used in a 25 µl reaction
- The activity of ***TRANSCRIPTME*** Reverse Transcriptase is inhibited by metal ion chelating agents (e.g. EDTA), inorganic phosphors, pyrophosphates and polyamines
- **Enzyme inactivation** should be carried out at 85°C for 5 min

Storage buffer

10 mM Tris-HCl (pH 8.0), 80 mM NaCl, 0.2 mM EDTA, 50% (v/v) glycerol

10x RT Reaction Buffer

500 mM Tris-HCl (pH 8.3), 750 mM KCl, 30 mM $MgCl_2$, 100 mM DTT

Troubleshooting

Problem	Possible cause	Solution
No cDNA synthesis or low efficiency synthesis	RNA has degraded	Store RNA at -70°C. Avoid subjecting the samples to repeated freezing and thawing. Place the RNA sample on ice or in a freezing rack immediately after thawing. Confirm the quality of RNA in a denaturating gel electrophoresis.
	Insufficient RNA for a reaction or low-quality RNA	Increase the quantity of RNA. Carry out RNA precipitation including the washing step, using 70% ethanol, in order to remove reaction inhibitors such as SDS, EDTA, sodium phosphate, spermidine, guanidine salts and formamide.
Unexpected bands in the electrophoretic image	Isolated RNA sample contains genomic DNA	Treat the contaminated RNA sample with DNase I.

TRANSCRIPTME

REVERSE TRANSCRIPTASE

Components	RT32-010 10 000 U	RT32-050 50 000 U	RT32-S **1000** U
TRANSCRIPTME Reverse Transcriptase (200 U/µl)	50 µl	5 x 50 µl	5 µl
10x RT Reaction Buffer	100 µl	5 x 100 µl	10 µl

Quality control

The absence of DNase and RNase activities has been confirmed using the relevant procedures. ***TRANSCRIPTME*** Reverse Transcriptase is >90% pure as judged by SDS polyacrylamide gel. In addition, the functional quality is tested by RT-PCR experiment.

Unit definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C, using poly(A) x oligo$(dT)_{12\text{-}18}$ as template primer.

Storage & shipping

Storage conditions

All components should be stored at -20°C in a freezer without a defrost cycle. When stored under optimum conditions, the reagents are stable until the expiry date.

Shipping conditions

Shipping on dry or blue ice.

For research use only

BLIRT S.A.
orders@blirt.eu | www.blirt.eu