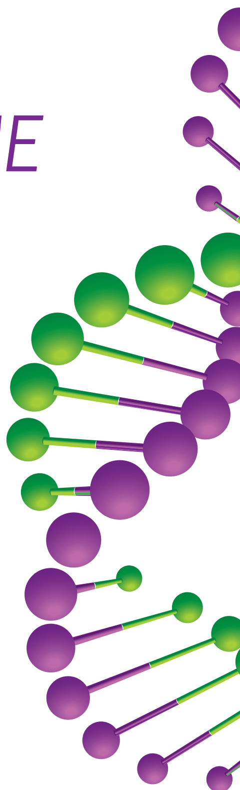
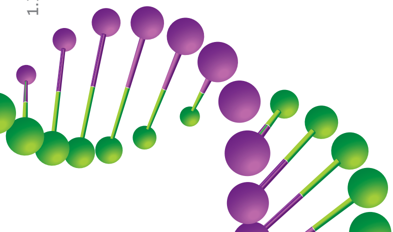
RT31-020, RT31-100

TRANSCRIPTME

RNA KIT



1.2020



blirt

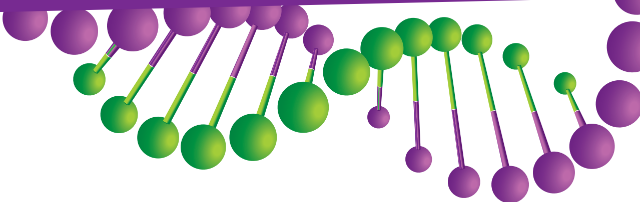
TRANSCRIPT *ME*
RNA KIT

The ***TRANSCRIPT****ME* **RNA Kit** is a system which includes all the necessary components for synthesizing first-strand cDNA, apart from the template total RNA or mRNA. The synthesized single-stranded cDNA is suitable for real-time quantitative RT-PCR applications. The ***TRANSCRIPT****ME* **RNA Kit** has been formulated to provide high yields of full-length cDNA product and to increase sensitivity in RT-qPCR. Starting material can range from 10 pg to 5 µg of total RNA.

The kit includes both the ***TRANSCRIPT****ME* **Enzyme Mix**, with a recombinant thermostable reverse transcriptase M-MuLV without RNase H activity and the ***RIBO****PROTECT* RNase Inhibitor. The optimal reaction buffer with a combination of **random hexamers** and **oligo(dT)$_{18}$ primers**, $MgCl_2$ and dNTPs mix is also provided for increased sensitivity.

TRANSCRIPT*ME* Reverse Transcriptase has increased thermal stability, which 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 or RNase H activity, which improves synthesis of a full-length cDNA, even from long mRNA templates, using random priming. The enzyme gives high yields of first strand cDNA up to 10 kb long.

Use of the recombinant RNase inhibitor ***RIBO****PROTECT* protects the RNA template from degradation. The enzyme selectively hydrolyzes the phosphodiester bonds of RNA only when it is hybridized to DNA. The **RNase H** does not degrade either single and double-stranded DNA or unhybridized RNA. This optional step can improve the sensitivity of subsequent RT-qPCR reaction since the PCR primers will bind more easily to the cDNA. Using of the **RNase H** is recommended only when it contributes to a full-length cDNA synthesis and increased yields of first-strand cDNA.



Features and advantages

- → High yields of full-length cDNA products (up to 10 kb)
- → Formulated to increase sensitivity in RT-qPCR and RT-PCR assays
- → Fewer pipetting steps – minimized contamination risk
- → Simultaneous cDNA synthesis with mRNA and rRNA templates
- → Starting material: 10 pg – 5 μg of total RNA or 10 pg – 500 ng of mRNA
- → Optimal reaction temperature: 50°C
- → The reverse transcriptase shows no RNase H or 3'→5' exonuclease activity, has supreme thermostability and is suitable for difficult RNA templates
- → RNase inhibitor protects the RNA template from degradation
- → Additional RNase H treatment may increase the sensitivity of the RT-qPCR reaction

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
RNA KIT

Protocol for the first strand cDNA synthesis

1. Add the 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 the reagent Master Mix without an RNA template is recommended). The reagents should be added in the following order:

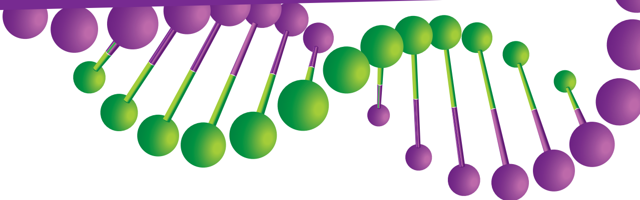
Reagent	Quantity
2x RT Master Mix	10 µl
***TRANSCRIPTME* Enzyme Mix**	2 µl
RNA	≤ 5 µg
Nuclease-free water	fill up to 20 µl

2. Mix gently and incubate at 25°C for 10 min.
3. Incubate at 50°C for 30 min.
4. Stop the reaction at 85°C for 5 min and **immediately** cool the sample on ice.

Optional step*: add 1 µl RNase H, incubate at 37°C for 20 min and stop the reaction at 85°C for 5 min.

* The RNase treatment may substantially increase the sensitivity of the RT-qPCR assays to the presence of traces of RNA.

5. 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 a 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.
- DNase I enzyme (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 the ***EXTRACT**ME* **TOTAL RNA KIT** (EM09.1) and ***EXTRACT**ME* **TOTAL RNA PLUS KIT** (EM11.1) for total RNA isolation from tissues and cell cultures.
- During RT-PCR reaction mixture preparation, keep all reagents of the kit 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 the RT-qPCR.
- The quantity of cDNA used when preparing PCR or qPCR reactions should not exceed 1/10 of the 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 ***TRANSCRIPT**ME* Reverse Transcriptase is inhibited by metal ion chelating agents (e.g. EDTA), inorganic phosphor, pyrophosphate and polyamines.

Quality control

The absence of DNase and RNase activity has been confirmed using the relevant procedures. Enzymes present in the ***TRANSCRIPT**ME* Enzyme Mix are > 90% pure as judged by SDS polyacrylamide gel electrophoresis. In addition, the functional quality is tested by RT-PCR experiment.

Troubleshooting

Problem	Possible cause	Solution
No cDNA synthesis or low efficiency synthesis	The RNA has degraded	Store RNA at -70°C. Avoid subjecting the samples to repeated freezing and thawing cycles. Place the RNA sample on ice or in a freezing rack immediately after thawing. Confirm the quality of the RNA quality 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.

TRANSCRIPT*ME*
RNA KIT

Components	RT31-020 20 rxns	RT31-100 100 rxns	RT31-S 3 rxns
TRANSCRIPT*ME* **Enzyme Mix** (1)	40 µl	5 x 40 µl	7 µl
2x RT Master Mix (2)	200 µl	5 x 200 µl	33 µl
RNase H *(E. coli)*	20 µl	5 x 20 µl	4 µl
Nuclease-free water	180 µl	5 x 180 µl	35 µl

1) Contains ***TRANSCRIPT****ME* Reverese Trascriptase and ***RIBO****PROTECT* RNase Inhibitor.
2) Contains RT-PCR buffer, oligo$(dT)_{18}$ primers, random hexamers, $MgCl_2$ and dNTPs mix.

Storage & shipping

Storage conditions

Store all components at -20°C. The stability of the reagents can be extended if stored at -70°C. The product is stable until the expiry date providing it is stored and used correctly.

Shipping conditions

Shipping on dry or blue ice.

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

blirt