INSTRUCTIONS FOR USE

Rons Ultimate cDNA Synthesis Kit

List of Components	Quantity REF 125800
10x DNase Incubation Buffer, 10x (yellow cap)	300 µl
DNase Enzyme Mix, 5 U/µl (green cap)	100 µl
Reverse Transcriptase, 200 U/µl (orange cap)	100 µl
5x RT Reaction Buffer, 5x (brown cap)	450 µl
dNTP Mix, 10 mM (transparent cap)	100 µl
Oligo$(dT)_{20}$ Primer, 100 µM (white cap)	100 µl
Random Hexamer Primer, 100 µM (pink cap)	100 µl
RNase Inhibitor, 40 U/µl (purple cap)	50 µl
RNase-free Water (blue cap)	1500 µl

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Introduction

Rons Ultimate cDNA Synthesis Kit contains all reagents required to reverse transcribe total RNA or mRNA into first-strand cDNA. The supplied reverse transcriptase has reduced RNase H activity and synthesises cDNA within 5 – 30 minutes. In addition, the kit contains a DNase enzyme mix with its incubation buffer for the optional removal of residual genomic DNA (gDNA) before cDNA synthesis, as well as Oligo(dT)$_{20}$ primer, random hexamer primer, dNTP mix, RNase inhibitor and RNase-free water.

Applications

The kit is intended for:

- First-strand cDNA synthesis from total RNA or purified mRNA
- Preparation of cDNA templates for subsequent PCR and qPCR (two-step RT-PCR)

Reverse transcription can be primed with Oligo(dT)$_{20}$ primer, random hexamer primer, a 1:1 mixture of both, or gene-specific primers.

Principle of the method

cDNA synthesis starts from a primer hybridised to the RNA template. Oligo(dT)$_{20}$ primer binds to the poly(A) tail of mRNA, random hexamer primer anneals at multiple positions of all RNA species, and gene-specific primers bind to a defined target sequence. Starting from the primer, the reverse transcriptase synthesises the complementary DNA strand from the supplied dNTPs. Owing to its reduced RNase H activity, the enzyme degrades the RNA strand of the RNA:cDNA hybrid only to a limited extent during synthesis, which supports the generation of long cDNA. The RNase inhibitor protects the RNA template from degradation. In the optional preceding step, residual gDNA is digested by the DNase enzyme mix, which is then inactivated at 95 °C. The cDNA obtained can be used directly as template in PCR or qPCR.

Product Specifications

Parameter	Specification
Reverse transcriptase	Reduced RNase H activity, 200 U/µl
RNA input (20 µl reaction)	Total RNA: 10 pg – 5 µg; purified mRNA: 10 pg – 500 ng
cDNA length	Up to 17 kb
Reaction time	5 – 30 minutes (standard: 10 minutes at 50 °C)
Primers supplied	Oligo(dT)$_{20}$ primer and random hexamer primer, 100 µM each; customer designed, gene-specific primers can be used
gDNA removal	Optional; DNase enzyme mix and incubation buffer included
Nuclease activity	No detectable endonuclease or exonuclease activity

Product use limitations

- For research use only. Not for use in diagnostic procedures.
- Use not more than 5 µl of the cDNA reaction in a subsequent application such as qPCR.
- For a one-tube one-step RT/ qPCR Master Mix, please ask for our Rons Ultimate One-Step RT-PCR Master Mix, REF 125610.

Warning and precautions

- Keep all components on ice during reaction set-up.
- Mix the reactions gently and spin them down briefly before incubation.
- Avoid repeated freeze-thaw cycles of the kit components and of the synthesised cDNA.

Transportation & Storage

- Store synthesised cDNA at -22 °C to -18 °C.

Storage	Temperature and Conditions
Long Term	-22 °C to -18 °C
Short Term	+2 °C to +8 °C
Shelf Life	24 months from date of manufacture

Handling Recommendations / Procedure

Prepare all reactions on ice. The procedure comprises an optional gDNA removal step (1.), the reaction set-up according to either the standard protocol (2.) or the extended protocol (3.), and the incubation described in the Reverse Transcription Protocol (4.).

1. Removal of genomic DNA (optional)

Residual gDNA can be co-amplified in subsequent PCR or qPCR and cause false-positive signals. Its removal is recommended if only RNA is to be analysed, in particular when the primers or probes of the downstream assay do not span an exon-exon junction.

a. Prepare the DNase reaction mix:

Component	10 µl Reaction	Final Concentration
10x DNase Incubation Buffer	1 µl	1x
DNase Enzyme Mix, 5 U/µl	1 µl	0.5 U/µl
RNA sample, variable	0.1 – 2 µg	variable
RNase-free Water	adjust to 10 µl	-

b. Incubate as follows:

Step	Time	Temperature
Digestion	5 – 10 minutes	37 °C
Inactivation	2 minutes	95 °C

c. Spin down the reaction and use it immediately for cDNA synthesis according to the Standard Protocol (2.), the Extended Protocol (3.) or place it on ice until further use.

2. Standard Protocol (recommended)

Combine the following components on ice, mix gently and continue with the Reverse Transcription Protocol (4.).

Component	20 µl Reaction	Final Concentration
RNA template or DNase-treated sample*	10 µl	10 pg – 5 µg per reaction*
Primer**	1 µl	5 µM**
5x RT Reaction Buffer	4 µl	1x
dNTP Mix, 10 mM	1 µl	500 µM
RNase Inhibitor (optional), 40 U/µl	0,5 µl	1 U/µl
Reverse Transcriptase, 200 U/µl	1 µl	10 U/µl
RNase-free Water	adjust to 20 µl final volume	-

* Total RNA: 10 pg – 5 µg; purified mRNA: 10 pg – 500 ng; alternatively, the reaction from the gDNA removal step (1).

** Oligo(dT)$_{20}$ primer, random hexamer primer or a 1:1 mixture of both. For gene-specific primers, a final concentration of 500 nM is recommended.

3. Extended Protocol

This protocol is recommended for RNA templates with pronounced secondary structure. Primer and RNA are heated together before the remaining components are added.

a. Prepare the primer/RNA pre-mix:

Component	10 µl Pre-Mix	Final Concentration
RNA template or DNase-treated sample*	variable, up to 9 µl	10 pg – 5 µg per reaction*
Primer**	1 µl	5 µM**
RNase-free Water	adjust to 10 µl	-

*, ** see footnotes of the Standard Protocol (2.). Final concentrations refer to the complete 20 µl reaction.

b. Incubate the pre-mix for 5 minutes at 70 °C and place it on ice immediately afterwards.

c. Prepare the final reaction mix, add it to the cooled pre-mix (final volume 20 µl), mix gently and continue with the Reverse Transcription Protoco (4.)l.

Component	10 µl Final Mix	Final Concentration
5x RT Reaction Buffer	4 µl	1x
dNTP Mix, 10 mM	1 µl	500 µM
RNase Inhibitor (optional), 40 U/µl	0.5 µl	1 U/µl
Reverse Transcriptase, 200 U/µl	1 µl	10 U/µl
RNase-free Water	adjust to 10 µl	-

4. Reverse Transcription Protocol

Step	Time	Temperature	Cycles
Reverse Transcription	10 minutes*	50 °C*	1 x
Heat Inactivation (optional)	2 minutes	95 °C	1 x

***** The required incubation time depends on the cDNA length. For long templates or templates that are difficult to transcribe, the incubation can be extended to 30 – 60 minutes and/or the temperature raised to 55 °C.

After reverse transcription, use the cDNA directly in PCR or qPCR or store it at -22 °C to -18 °C.

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number	LOT	Batch code
	Temperature limit		Follow the instructions for use
	Manufacturer		Use-by date
	Date of manufacture	Σ	Contains sufficient for <n> tests
	Caution		Keep away from sunlight

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