

Ultimate cDNA Synthesis Kit

Description: This cDNA Synthesis Kit contains all necessary components for an efficient reverse transcription of RNA or mRNA into cDNA. The Kit includes a Reverse Transcriptase which reduced RNaseH activity for fast and specific generation of cDNA in 5 – 30 minutes. An additional enzyme mix can be used for the efficient removal of gDNA.

With the Ultimate cDNA Synthesis Kit amplicons up to 17 kb can be amplified with an RNA input of 10 µg per reaction. All reagents are free of detectable endonuclease- & exonuclease activity.

Storage: -18 °C to - 22 °C for long term, + 2 to + 8 °C for short term. Protect from light. Avoid freeze-thaw cycles of the reagents and the final product.

REF 125800	Stock Concentration	colour
10x DNase Incubation Buffer	10x	yellow
DNase Enzyme Mix	5 U/µl	green
Reverse Transcriptase	200 U/µl	orange
5x RT Reaction Buffer	5x	brown
dNTP Mix	10 mM	transparent
Oligo(dT) ₂₀ Primer	100 µM (100 pmol/ µl)	white
Random Hexamer Primer	100 µM (100 pmol/ µl)	pink
RNase Inhibitor	40 U/µl	purple
RNase-free Water	--	blue

Application: Synthesis of cDNA from RNA templates.



- Please keep all components on ice while reaction set-up.
- Do not use more than 5 µl for downstream applications like qPCR.
- For a one-tube one-step RT/ qPCR Master Mix, please ask for our Ultimate One-Step RT-PCR Master Mix, REF 125610

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1. Recommended Standard Protocol

Add all reagents according to the table below, mix gently and continue with the protocol in 3.

Component	20 µl Reaction	Final Concentration
RNA template or DNase-digested sample*	10 µl	10 pg to 5 µg/reaction*
Primer**	1 µl	5 µM**
5x RT Reaction Buffer	4 µl	1x
dNTP-Mix	0.5 µl	500 µM
RNase Inhibitor (optional)	1 µl	1 U/µl
Reverse Transcriptase	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

** use either Oligo(dT)Primer or Random Primer or a 1:1 mixture of both; for gene specific primers we recommend to use 500 nM final primer concentration

2. Extended Protocol

This protocol is recommended for RNA targets with complex secondary structures.

2.1 Prepare the following pre-mix:

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

* Total RNA: 10 pg – 5 µg; purified mRNA: 10 pg – 500 ng

** use either Oligo(dT)Primer or Random Primer or a 1:1 mixture of both; for gene specific primers we recommend to use 500 nM final primer concentration

2.2 Incubate the pre-mix for **5 min at 70 °C**, then put on ice immediately.

2.3 Prepare the final reaction mix according to the table below, mix gently and continue with the protocol in 3.

Component	20 µl Reaction	Final Concentration
5x RT Reaction Buffer	4 µl	1x
dNTP-Mix	1 µl	500 µM
RNase Inhibitor (optional)	0.5 µl	1 U/µl
Reverse Transcriptase	1 µl	10 U/µl
RNase-free Water	adjust to 10 µl volume	--
add this mix to pre-mix with Primer/RNA for final reaction volume of 20 µl		

3. Reverse Transcription Protocol

Step	Time	Temperature
Reverse Transcription	10 minutes	50 °C
Optional: Heat Inactivation	2 minutes	95 °C

Difficult and long templates may require a time of 30 – 60 min to increase yield and cDNA length and an increase of temperature to 55 °C.

4. gDNA Removal Protocol (optional)

Residual genomic DNA can negatively impact the results of cDNA synthesis and subsequent applications. Therefore, digestion of gDNA improves the quality of cDNA synthesis. It is recommended for samples where only RNA is to be analysed after the reaction and for following PCR/qPCR assays where the used primers/probes are not intron or exon spanning.

4.1 Reaction Mixture

Prepare DNase I reaction mix according to the table below and continue with the digestion protocol

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

4.2 Digestion Protocol

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

Spin the mix down and use immediately for cDNA synthesis with 1. Standard Protocol or 2. Extended Protocol.