

Ultimate One-Step RT-PCR Master Mix

Description: The Ultimate One-Step RT-PCR Master Mix is an advanced mixture for probe-based One Tube RT-qPCR (Reverse Transcription and Real-Time PCR). This Master Mix contains all necessary enzymes for RT and qPCR in an optimized buffer system with dNTPs and MgCl₂. Pipetting steps and contaminations can be minimized by only pipetting primer, probe and sample RNA to the mix. This Master Mix is suitable for amplification of difficult templates and Multiplex reactions.

Concentration: 2x

Storage: -18 °C to -22 °C for long term, +2 to +8 °C for short term in the dark

REF	125610	colour
Ultimate One-Step RT-PCR Master Mix	100 reactions	white

Application: One-Step and One-Tube RT-qPCR for analysis of RNA samples with different kind of qPCR probes.

Quality Control: All Master Mixes are free of detectable endonuclease and exonuclease activity. Each lot is tested in a probe-based assay with RNA input.



- Some PCR cyclers require ROX as reference dye. If you want ROX included in this Master Mix, please do not hesitate to contact us.
- For cDNA Synthesis only, we recommend our Ultimate cDNA Synthesis Kit, REF 125800
- After thawing, invert the Master Mix tube carefully several times.
- Do not vortex the Master Mix to avoid damaging the enzyme.

For research use only. Not approved for use in clinical or in-vitro diagnostics.

Recommended Standard Protocol:

Component	20 µl Reaction	Final Concentration
2x Ultimate One-Step RT-PCR MM	10 µl	1 x
Primer Forward/ Reverse	Variable	400 nM
Probe	Variable	400 nM
Template RNNA	Variable	0.1 – 100 ng per reaction
PCR Water	adjust to 20 µl final volume	--

Reaction set-up is possible at room temperature. After mixing by pipetting, centrifuge briefly and place the tube into a standard thermocycler or Real Time PCR equipment. When using one of the following Cycling Protocols, please be aware that the conditions also depend on your primer, probe and qPCR cyler and therefore adjustments might be necessary.

Recommended Thermocycler Protocol

Step	Time	Temperature	Cycles
Reverse Transcription	10 minutes	50 – 55 °C	1 x
Initial Denaturation (Inactivation of Reverse Transcriptase)	2 - 3 minutes	92 – 95 °C	1x
Denaturation	5 -10 seconds	92 – 95 °C	25 – 40 x
Annealing/ Extension combined	10 seconds	55 – 68 °C*	

Recommended Thermocycler Protocol - Ultra Fast

Step	Time	Temperature	Cycles
Reverse Transcription	5 minutes	50 – 55 °C	1 x
Initial Denaturation (Inactivation of Reverse Transcriptase)	1 minute	92 – 95 °C	1x
Denaturation	1 second	92 – 95 °C	25 – 40 x
Annealing/ Extension combined	1-5 seconds	55 – 68 °C*	

* Depends on primer, the optimal annealing temperature is usually 2 – 5°C below the primer melting temperature

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