Check the product label for actual catalog number, lot and expiry date.

1Step RT PCR Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTK0201	100 r of 50 µl	2 x 1.25 ml - 1Step RT PCR Mastermix, 2X 2 x 0.125 ml - RT2 Mix, 20X 3 x 1 ml - PCR Water	1X 1Step RT PCR Mastermix contains hot-start Taq DNA Polymerase, 0.25 mM dNTPs, 3 mM $MgCl_2$, enhancers, stabilizers. RT2 Mix is a 20X concentrated blend of reverse transcriptase and RNase inhibitor

Storage *In the dark at -20°C.*

APPLICATIONS

- One step RT-PCR
- RT-PCR of complex GC/AT rich templates
- Fast RT-PCR
- TA cloning

BENEFITS

- Easy to use combination of the RT mix with the RT-PCR mastermix allowing for reverse transcription and subsequent PCR in one tube
- RT Mix contains RNase inhibitor and thermostable reverse transcriptase (up to 55°C) allowing for high cDNA yields
- RT PCR Mastermix allows for sensitive low copy number targets detection due to proprietary hot-start
- The combination of both mixes provides high yields under standard and fast cycling conditions and from complex GC/AT rich templates

PRODUCT DETAILS

highQu 1Step RT PCR Kit combines the 20X RT enzyme mix for efficient reverse transcription and the 2X PCR mastermix for subsequent amplification of cDNA in the same tube.
RT2 Mix, 20X is a blend of the engineered MMuLV, stable at 40-55°C and allowing for high yields of long transcripts with an efficient Ribonuclease inhibitor protecting the template RNA from RNases.
The resulting cDNA is then amplified by the 1Step RT PCR Mastermix, 2X. The mastermix contains our proprietary Hot Start Taq DNA Polymerase. The activity at room temperature is blocked by small molecular inhibitor. Enzyme becomes active only after heating what allows for highly specific and extremely sensitive amplification, no primer dimer formation and no background.

PRODUCT DETAILS

In combination with the optimized buffer the enzyme provides higher success rates in demanding PCR applications like amplification of complex or longer templates and fast cycling.
Hot Start Taq DNA Polymerase has the same PCR accuracy like Taq DNA Polymerase, and produces A-tailed products suitable for ligating into TA cloning vectors.

For the maximum convenience the Kit includes even the PCR water to set up the reaction, so the only thing you need to take care is the high quality RNA template.

PROTOCOL

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation and to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Include a no-template control, no RT2 Mix control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Perform cDNA synthesis 10 min at 45°C, 20 min for >1 kb, increase temperature to 55°C for complex templates.
- The longer the amplicon, the longer the extension time: Use 15 sec/kb extension for amplicons of <3 kb.
- Use 40-60 sec/kb extension for amplicons of 5-10 kb.
- Run an annealing temperature gradient from 58°C to 65°C to choose the best specificity conditions.

✓ Prepare a 50 µl reaction:

Rev. & For. Primers	0.2-0.4 µM final each (2 µl of 10 µM each)
Total RNA *or* mRNA	1 pg to 1 µg *or* >0.01 pg
PCR Water	to 22.5 µl
1Step RT PCR Mastermix, 2X	25 µl
RT2 Mix, 20X	2.5 µl

✓ Mix gently, avoid bubbles.

✓ Place into the instrument set like:

Reverse transcription	1 cycle: 45 -55°C – 10 to 20 min
Initial denaturation	1 cycle: 95°C – 2 min
Denaturation	40 cycles: 95°C - 10 sec
Annealing	40 cycles: 60-65°C – 10 sec
Extension	40 cycles: 72°C –30-60 sec (15 sec/kb)

✓ Store probes for short time on ice, for long at -20°C.

IN VITRO RESEARCH USE ONLY

ORDERING
T: +49 7250 33 13 401
F: +49 7250 33 11 413
order@highQu.com
www.highQu.com

SALES
T: +49 7250 33 13 401
F: +49 7250 33 11 413
sales@highQu.com

TECHNICAL SUPPORT
T: +49 7250 33 13 401
F: +49 7250 33 11 413
tech-support@highQu.com