



The ENZYME Company

One tube RT-PCR Kit “Hot-Start” for qPCR

The RT-qPCR kit is designed especially for simple and effective one-tube RT-qPCR. The reverse transcription step is directed by M-MuLV reverse transcriptase, as soon as cDNA is synthesized, a chemically modified “ Hot-Start” Taq DNA polymerase, is activated by heating up the reaction 10 min at 95°C. As soon as the Polymerase is activated, regular PCR procedure is performed.

The kit for one tube RT-qPCR contains:

1. qPCR Reaction buffer qA (5x) (MgCl₂ 15mM)
2. dNTPs 5mM
3. Mixture of the enzymes
4. DNase-RNase free water

Storage at – 20 °C

Catalog #	Pack size
122305	50 rcs
122325	500 rcs

Protocol for one tube RT-PCR:

Mix thoroughly in the PCR-tube:

- | | | |
|----------------------------|---------|-----------------|
| 1. Total RNA | | 1-5 µl (1–10ng) |
| 2. Reaction buffer qA (x5) | | 5 µl |
| 3. dNTP 5mM | | 2,5 µl |
| 4. Primers: | | |
| | reverse | 20 pmoles |
| | forward | 10 pmoles |
| 5. Enzymes mix | | 1 µl |
| 6. Water | | up to 25 µl |

After intensive mixing, centrifuge briefly and place the tube into the thermocycler. We recommend to use the following program:

1. 65°C – 5 min
2. 4°C – 3 min
3. 42°C – 30min (15-30min)
4. 95°C – 10min
5. 25-35 amplification cycles
6. 72 °C – 5 min
7. 10 °C – storage

Step 3 (reverse transcription) can be performed longer (up to 1 hour) in the case of long or highly structured template. The temperature of amplification steps should be optimized for the particular amplicon.

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