Xpert One-Step RT-PCR Kit

#GK64.0100 (100 rxns)
(FOR RESEARCH ONLY)

Product: Using gene-specific primers (GSP), the Xpert One-Step RT-PCR Kit allows for first-strand cDNA synthesis and subsequent PCR in a single-tube reaction procedure, decreasing contamination risk and reducing hands-on time considerably. The kit consists of a RTase mix and a Fast PCR Mastermix. The RTase mix comprises genetically modified thermostable MMLV reverse transcriptase with improved synthesis efficiency, lacking any RNase H activity, and an advanced RNase inhibitor to impede RNA degradation. The Fast PCR Mastermix contains all other required components, including a fast hot-start DNA polymerase for improved speed, sensitivity and specificity, $MgCl_2$, dNTPs, enhancers and stabilizers.

The Xpert One-Step RT PCR Kit can be used with used with any RNA template, including mRNA, viral RNA and total RNA, as the RTase is not inhibited by rRNA or tRNA. The Kit provides a robust RT-PCR performance that requires minimal or no optimization. PCR products contain 3´A-overhang and are thus suitable for TA cloning

Applications: RT-PCR

Contents: The Xpert One-Step RT-PCR Kit contains sufficient reagents for 100 reactions of 25µl. The PCR Mastermix contains, besides the hot-start Taq Polymerase, 6mM MgCl2, 2mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or MgCl2, as the buffer composition was already optimized for sensitivity, specificity and yield.

Component	GK64.0100
RTase Mix	125 µl
Fast PCR Mastermix	1.25 ml

Samples: total RNA: 1pg - 1µg, mRNA: >0.01pg

Storage: Store all components at -20°C

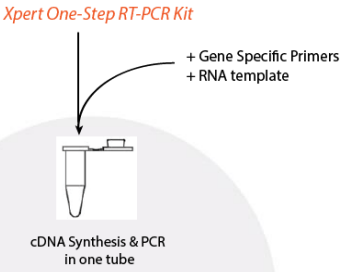
Prior to use:

briefly vortex PCR Mastermix.

Usage:

1. Mix the following components in a RNase-free microtube:

Component	Volume[1]
Fast PCR Mastermix	12.5 µl
Forward GSP (10µM)	1.0 µl
Reverse GSP (10µM)	1.0 µl
template RNA	see note 2
RTase Mix	1.25 µl
RNase-free water	up to 25 µl



[1] The reaction can be easily scaled-up or down by changing the volumes proportionally.
[2] Template RNA: in case of total RNA 1pg- 1µg, in case of mRNA > 0.01pg
[3] The mixture already contains optimized amounts of $MgCl_2$ and dNTPs. It is not recommended to add further components.
[4] It is recommended to include a no-RTase control for each experiment.

2. Using a thermocycler and set-up the following cycling conditions:
   a. 10-15 min at 45°C (cDNA synthesis)
   b. 3 min at 95°C (hot-start)
   c. 30-40 cycles of 10 seconds at 95°C, 10 seconds at Ta*), 15sec/kb at 72°C
   d. 1 min at 72°C (final extension), then put on hold at 4°C.

3. Either use or analyze PCR product immediately or store at -20°C.

Notes

1. The RNA sample should be completely free of contaminating genomic DNA.
2. Larger amounts of template RNA (up to 5µg total RNA) may be added for increased cDNA yield, however, complete reverse transcription may be compromised and is thus not guaranteed.
3. For most applications, cDNA synthesis (step 2a) should be carried out at 45°C. In case of targets with high secondary structure, synthesis may be optimized by carrying out at 55°C
4. *) Ta depends on the Tm of both GSP, which in turn depend on the buffer composition. Ideally, primers should have a predicted Tm of about 60°C. In general, using a Ta of 1-3°C above the Tm of the primer with the lowest Tm is a good starting point. It is highly recommended to determine optimum annealing temperature experimentally by performing a temperature gradient.
5. The optimal extension time depends on the size of the amplicon as well as on the complexity of the template. 15 seconds per kb is recommended for the PCR amplification of DNA between 1 and 3kb.