TECHNICAL DATA SHEET

Classic++™ 1-Step RT-PCR Kit

Catalog Numbers: 31-5220-0050R
31-5220-0100R

PRODUCT INFORMATION

Contents: 31-5220-0050R (50 rxns)
Classic++™ 1-Step Master Mix (2X): 1 x 1.25 mL
M-MLV RTase / RNase Inhibitor Mix (20X): 1 x 125 µL

31-5220-0100R (100 rxns)
Classic++™ 1-Step Master Mix (2X): 2 x 1.25 mL
M-MLV RTase / RNase Inhibitor Mix (20X): 2 x 125 µL

Use By: 6 months from date of receipt

DESCRIPTION

The Classic++™ 1-Step RT-PCR Kit's advanced reagents allow for efficient cDNA synthesis and PCR in a single tube. Included in the Master Mix is Classic++™ Hot Start *Taq* DNA polymerase, a next generation thermostable polymerase of recombinant origin that possess 5'→3' polymerase activity. It also contains two proprietary Classic++™ Hot Start mAbs (monoclonal antibodies) which are used to specifically block polymerase activity below 70°C, allowing for convenient room temperature reaction set up. Following successful reverse transcription of RNA target molecules, DNA polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 94-95°C for two minutes. The blocking of polymerase activity prior to denaturation of template improves yields by minimizing or eliminating primer dimer formation and non-specific amplification. In addition, a proprietary 20X M-MLV Reverse Transcriptase (RT) molecule blended with an optimized RNase inhibitor is included in a separate tube. The enzyme can synthesize cDNA at a temperature range of 45–55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Our M-MLV RT is not significantly inhibited by ribosomal and transfer RNA, making it ideal to synthesize cDNA from total RNA.

RT-PCR (reverse transcription-polymerase chain reaction) is used to convert and amplify a single-stranded RNA template to yield abundant double-stranded complementary DNA (cDNA) product. One-step RT-PCR is a variation on standard RT-PCR in which all components are mixed in one tube prior to starting the reactions. This approach offers simplicity and convenience and minimizes the possibility for contamination.

The Classic++ 1-Step RT-PCR Kit is designed for the sensitive, reproducible, end-point detection and analysis of mRNA or total RNA molecules by RT-PCR. Our convenient formulation allows for high speed, accurate and reproducible cDNA synthesis and subsequent PCR amplification in a single tube using gene-specific primers, starting from a variety of target RNA templates.

STORAGE

Store kit at -20°C upon arrival and limit exposure to light. The kit may undergo up to 30 freeze/thaw cycles without loss of activity. When stored correctly this kit will retain activity for up to 6 months. This product can be stored at 4°C for up to 1 month.

BIOLOGICAL SOURCE

The Classic++ Hot Start *Taq* polymerase is a single recombinant polypeptide of bacterial origin having a molecular weight of ~94 kDa, originally derived from the YT-1 strain of *Thermus acquaticus*. Classic++ Hot Start mAbs are of murine origin and are reactive with select epitopes found within recombinant forms of the YT-1 strain of *Thermus acquaticus*. Tonbo's Reverse Transcriptase is a proprietary version of M-MLV RT that has been genetically engineered to reduce RNase H activity and provide increased thermal stability.

APPLICATION NOTES

Master Mix: Classic++ 1-Step Master Mix (2X) contains Classic++ Hot Start *Taq* DNA Polymerase, Classic++ Hot Start mAbs, 6 mM $MgCl_2$ and 2 mM dNTPs in a buffer that includes a proprietary mix of stabilizers and enhancers. This Master Mix has been rigorously developed for optimal PCR success rate, yield and efficiency. We do not recommend introducing additional $MgCl_2$ or enhancers to the reaction mix.

M-MLV RTase / RNase Inhibitor Mix: Please refer to the reaction mix preparation step under Reaction Setup/Quick Protocol. Do not reduce the recommended volume per reaction as using an incorrect amount of RTase/RNase inhibitor will result in loss of sensitivity. If the final reaction volume is less than 50 µL, adjust the volume of 20X RTase accordingly.

Primers: We recommend that primers have a predicted melting temperature of approximately 60°C using default Primer 3 settings (http://bioinfo.ut.ee/primer3/). For each reaction, a final primer concentration of 0.2 - 0.6 µM is suggested.

Template: We recommend using between 1 pg - 1 µg of total RNA per reaction. For mRNA, use at least 0.01 pg per reaction.

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Reverse Transcription Reaction: Incubation for 10 minutes at 45°C should work well for most applications, however one may consider the following adjustments as appropriate. Increase the incubation time to 20 minutes for amplicons larger than 1 kilobase (kb). Temperature can be increased up to 55°C in cases where regions of interest have a high degree of secondary structure (>65% GC).

Annealing Temperature: It is preferable to generate a temperature gradient in order to empirically determine the optimal annealing temperature for the reaction. Otherwise, one can start with an annealing temperature of 55°C and, if non-specific products are observed, increase in 2°C increments (up to maximum 65°C) until an optimal temperature is reached.

Extension Temperature: We observe optimal extension at 72°C. Extension time depends on both the template complexity and amplicon length. For amplicons between 1 - 3 kilobases (kb) from eukaryotic DNA, we recommend 15 seconds per kb.

REACTION SETUP / QUICK PROTOCOL

1. Ensure all components are thawed and mixed well. Set up reactions on ice. Refer to Table 1 for reaction preparation. If preparing multiple reactions, assemble all common components into a master reaction mix. If working with final reaction volumes less than 50 µL, scale component volumes accordingly.
2. Optional: We recommend including a no-RTase control reaction (do not add M-MLV RTase / RNase Inhibitor Mix) which will act as a control for the presence of contaminating genomic DNA in the reaction. A no-template control (do not add template RNA) can also be set up as a control for the presence of contaminating genomic DNA in the enzyme/primer mixes.
3. As applicable, transfer the recommended volume of master reaction mix, primers and sample template RNA to individual PCR tubes or plates, seal and spin briefly to mix. Refer to the cycling conditions (Table 2) to perform the reaction.

Table 1. Reaction Preparation

Reagent	50 µL reaction	Final Concentration	Notes
1-Step Master Mix (2X)	25 µL	1x	
Forward Primer (10 µM)	2.0 µL	400 nM	See above for optimal primer design
Reverse Primer (10 µM)	2.0 µL	400 nM	
RTase Mix (20X)	2.5 µL	1x	Correct volume is critical Do not reduce
Template RNA	1 pg to 1 µg total RNA >0.01 pg mRNA	variable	
Nuclease free dH_2O	Up to 50 µL final volume		

Table 2. Cycling Conditions

Cycles	Temperature	Time	Notes
1	45°C - 55°C	10 minutes	Reverse transcription, 45°C is recommended for most applications. 55°C should be used only when amplicon contains regions of high secondary structure.
1	95°C	2 minutes	Polymerase activation
40	95°C 60°C - 65°C 72°C	10 seconds 10 seconds 30 to 60 seconds	Denaturation Anneal Extension (15 seconds per kb)

TECHNICAL SUPPORT

Please provide the following information to support@tonbobio.com for troubleshooting and technical support:

- Catalog and batch numbers
- Reaction set-up (master mix)
- Cycling conditions
- Amplicon size
- Screen shots of gel images
- Detailed description of the issue

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