MyGo

REAGENTS FOR RESULTS

MyGo 1-Step RT-PCR Kit

Cat. No. 7468 | 8990 | 3321

Component	500 Units (7468)	2000 Units (8990)	4000 Units (3321)
2x MyGo 1-Step Mix	1 x 1.25ml	2 x 1.25ml	10 x 1.25ml
20x RTase with RNase inhibitor	1 x 125µl	2 x 125µl	10 x 125µl

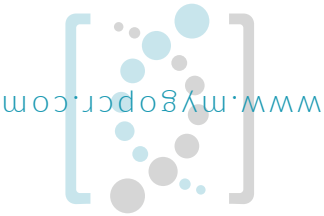
This product is for research use only

1. STORAGE

Store all components at -20°C with minimal exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit may be stored at 4°C for short term use (1 month). The kit can go through up to 30 freeze/thaw cycles with no reduction in performance.

2. TECHNICAL ASSISTANCE

If you have any questions, or experience any difficulties with MyGo 1-Step RT-PCR Kit, please email reagentsupport@mygopcr.com, providing full details including amplicon size, reaction setup, cycling conditions and screen shots of gel images.



3. DESCRIPTION

MyGo 1-Step RT-PCR Kit contains all the reagents required for rapid and sensitive cDNA synthesis and subsequent PCR in a single tube. The kit includes a thermostable reverse transcriptase and 2x MyGo 1-Step Mix containing MyGo HS Taq DNA Polymerase, dNTPs, and $MgCl_2$ optimised to give the best results under challenging conditions such as high GC or low-copy RNA targets.

The kit uses a thermostable modified Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase optimised for reliable cDNA synthesis over a wide dynamic range of input RNA. The enzyme is provided preblended with RNase inhibitor to prevent degradation of RNA by contaminating RNase.

MyGo 1-Step Mix uses antibody-mediated hot start to provide highly specific and sensitive 1-Step RT-PCR. MyGo HS Taq DNA Polymerase is inactive at ambient temperatures, preventing the formation of primer-dimers and mis-primed products with the convenience of room temperature setup.

4. IMPORTANT NOTES

4.1 2x MyGo 1-Step Mix: The 2x mix contains MyGo HS Taq DNA Polymerase, 6mM $MgCl_2$, 2mM dNTPs, enhancers and stabilizers. The buffer system has been optimised for the best PCR results and we do not recommend adding further enhancers or $MgCl_2$ to the reaction.

4.2 20x RTase: The 20x RTase is provided preblended with RNase inhibitor and it is essential to use the correct volume per reaction. Using the incorrect volume will result in loss of sensitivity.

4.3 Template: Use 1pg to 1µg total RNA per reaction, or a minimum of 0.01pg mRNA per reaction.

4.4 Primers: We recommend using primer design software Primer 3 (http://frodo.wi.mit.edu/primer3/). Primers should have a melting temperature (Tm) of approximately 60°C. The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

4.5 Reverse Transcription: Incubating with a temperature of 45°C for 10 minutes is recommended for the majority of applications. Use an incubation temperature of 55°C only when the amplicon of interest contains regions of high secondary structure. For amplicons above 1kb, increase the incubation time to 20 minutes.

4.6 Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. As an alternative, you can use a 55°C annealing temperature then increase in 2°C increments if non-specific products are present.

4.7 Extension: The recommended extension temperature is 72°C. The optimal extension time will depend on amplicon length and complexity of template. We recommend 15 seconds per kilobase (kb) for amplification from eukaryotic DNA (amplicons between 1kb and 3kb).

5. REACTION SETUP

5.1 Gently vortex 2x MyGo 1-Step Mix then prepare a master mix as follows. We recommend you also set up a no-RTase control:

Component	50µl reaction	Final concentration	Notes
2x MyGo 1-Step Mix	25µl	1x	
Forward primer (10µM)	2.0µl	400nM	See 4.4 above
Reverse primer (10µM)	2.0µl	400nM	
20x RTase	2.5µl	1x	Correct volume is critical. This should not be reduced or increased.
Template RNA	1pg to 1µg total RNA >0.01pg mRNA	variable	
PCR grade water	Up to 50µl total volume		

5.2 Cycle using the following conditions:

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