MyGo

REAGENTS FOR RESULTS

MyGo cDNA Synthesis Kit

Cat. No. 8304 | 3810

Component	25 Reactions (8304)	100 Reactions (3810)
5x cDNA Synthesis Mix	100µl	400µl
20x RTase (with RNase inhibitor)	25µl	100µl

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 cDNA Synthesis Kit, please email reagentsupport@mygopcr.com, providing full details of experimental conditions.

3. DESCRIPTION

MyGo cDNA Synthesis Kit is an easy-to-use, 2-tube system containing all the reagents required for rapid, sensitive and unbiased cDNA synthesis from total RNA or mRNA. The kit includes a thermostable reverse transcriptase and a 5x cDNA Synthesis Mix containing anchored oligo(dT), random hexamers, dNTPs, and $MgCl_2$ optimised for the generation of cDNA for use in real-time PCR.

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.

The MyGo cDNA Synthesis Kit is ideal for reverse transcription of many different RNA templates, including GC-rich templates and RNAs with high levels of secondary structure. The kit can be used with 4.0pg to 0.1µg total RNA or 0.2pg to 2.0µg of oligo(dT) purified mRNA.

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4. IMPORTANT NOTES

4.1 **5x cDNA Synthesis Mix:** The 5x mix contains anchored oligo(dT), random hexamers, 15mM $MgCl_2$, 5mM dNTPs, enhancers and stabilizers. The buffer mix has been optimised for the generation of cDNA for downstream real-time PCR analysis and we do not recommend adding further enhancers or $MgCl_2$ to the reaction.

4.2 **Template:** The kit can be used with 4.0pg to 0.1µg total RNA or 0.2pg to 2.0µg of oligo(dT) purified mRNA.

4.3 **Incubation temperature:** For the majority of applications (GC content less than 65%) we recommend incubating at 42°C for 30 minutes. Where regions of interest contain high secondary structure (GC content greater than 65%), an incubation temperature of up to 55°C can be used.

4.4 **Real-time PCR setup:** We recommend using 4.0µl of cDNA per 20µl qPCR reaction.

5. REACTION SETUP

5.1 Thaw the 5x cDNA Synthesis Mix and briefly vortex.

5.2 Prepare a master mix using the following table, making sure to add reagents in the sequence listed:

Component	20µl reaction	Final concentration	Notes
5x cDNA Synthesis Mix	4.0µl	1x	
20x RTase	1.0µl		Add before total RNA
Total RNA or Oligo(dT) purified mRNA	Xµl Total RNA: 4.0pg to 0.1µg Oligo(dT) purified mRNA: 0.2pg to 2.0µg		Variable
PCR grade water	Up to 20µl total volume		

OPTIONAL NO RT CONTROL SETUP

5.3 Prepare a master mix using the following table, making sure to add reagents in the sequence listed:

Component	20µl reaction	Final concentration	Notes
5x cDNA Synthesis Mix	4.0µl	1x	
Total RNA or Oligo(dT) purified mRNA	Xµl Total RNA: 4.0pg to 0.1µg Oligo(dT) purified mRNA: 0.2pg to 2.0µg		Use an equal amount of RNA as in 5.2 above
PCR grade water	Up to 20µl total volume		

INCUBATION

5.4 Incubate at 42°C for 30 minutes.

ENZYME DENATURATION

5.5 Incubate at 85°C for 10 minutes to denature RTase.