MyGo

REAGENTS FOR RESULTS

MyGo Probe 1-Step No ROX

Cat. No. 6245 | 9202 | 6355

Component	100 rxns (6245)	300 rxns (9202)	1200 rxns (6355)
2x MyGo Probe 1-Step No ROX	1 x 1ml	3 x 1ml	12 x 1ml
20x RTase (contains RNase inhibitor)	1 x 100µl	3 x 100µl	12 x 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 Probe 1-Step, please email reagentsupport@mygopcr.com, providing full details including amplicon size, reaction setup, cycling conditions and screen shots of amplification traces and melt profiles.

www.mygopcr.com

3. DESCRIPTION

MyGo Probe 1-Step contains all the reagents required for rapid and sensitive cDNA synthesis and subsequent real-time PCR in a single tube.

The kit and has been designed for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. It includes a thermostable reverse transcriptase and 2x MyGo Probe 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 Probe 1-Step Mix uses antibody-mediated hot start to provide highly specific and sensitive amplification. 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 Instrument compatibility

Some real-time PCR instruments require the use of ROX as a fluorescent passive reference to correct for optical artefacts. Generally, modern instruments do not require the use of passive fluorescent references. The MyGo instruments do not require the use of ROX as a passive reference. Please check our ROX Selection Table at mygopcr.com to determine which ROX concentration your instrument requires.

4.2 Amplicon length and primer design

Amplicon lengths of between 80bp and 200bp should be used for the highest efficiency under fast cycling conditions. Amplicons should not exceed 400bp. The shorter the amplicon length, the faster the reaction can be cycled. 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.

4.3 Template concentration:

As target copy number will vary, it is important to select the correct template concentration to correctly quantify the target sequence. A good concentration will display clear separation between amplification curves (Figure 1). At lower template concentrations, the amplification curves will begin to group together and Ct values will not fit the standard curve (Figure 2).

Figure 1

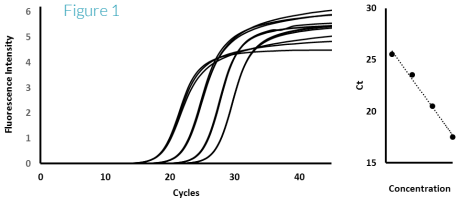
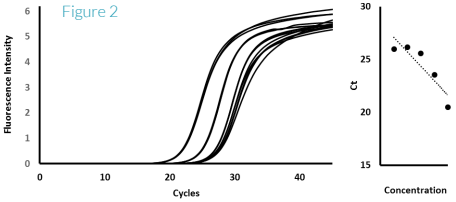


Figure 2



5. REACTION SETUP

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

Component	20µl reaction	Final concentration	Notes
2x MyGo Probe 1-Step Mix	10µl	1x	
Forward primer (10µM)	0.5µl	250nM	See 4.2 above
Reverse primer (10µM)	0.5µl	250nM	
Probe (10µM)	0.4µl	200nM	
20x RTase (contains RNase inhibitor)	1.0µl	1x	We recommend 1.0µl. Using 2.0µl will improve Ct but may increase primer-dimers
Template DNA	10pg to 100ng total RNA >0.01pg mRNA	Variable	See 4.3 above
PCR grade water	Up to 20µl total volume		

5.2 Program the instrument as follows, acquiring data on the appropriate channel:

Cycles	Temperature	Time	Notes
1	45°C to 55°C	10min	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	2min	Polymerase activation
40	95°C 60°C to 65°C	10 seconds 20-30 seconds	Denaturation Anneal/Extension: Do not exceed 30 seconds or use temperatures below 60°C
Melt analysis	Refer to instrument instructions		Optional (available for hybridisation probes only)