



REAGENTS FOR RESULTS

MyGo Green 1-Step High ROX

Cat. No. 8705 | 5235 | 5015

Component	100 rxns (8705)	300 rxns (5235)	1200 rxns (5015)
2x MyGo Green 1-Step High 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 Green 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.

3. DESCRIPTION

MyGo Green 1-Step contains all the reagents required for rapid and sensitive cDNA synthesis and subsequent real-time PCR in a single tube. The kit includes a thermostable reverse transcriptase and 2x MyGo Green 1-Step Mix containing MyGo HS Taq DNA Polymerase, dNTPs and MgCl₂ 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 Green 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.

The mix includes an intercalating dye which does not inhibit PCR, allowing accurate and reliable 1-step real-time RT-PCR without the use of sequence specific probes.



4. IMPORTANT NOTES

4.1 Instrument compatibility

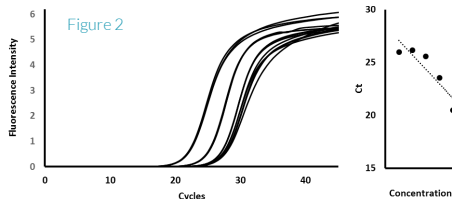
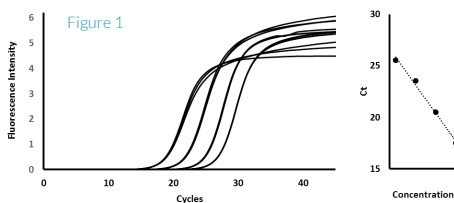
Some real-time PCR instruments require the use of ROX as a fluorescent passive reference to correct for optical artefacts. Generally, modern instruments (including MyGo instruments) do not require the use of passive fluorescent references. This kit contains ROX in case you want to use the kit with instruments that do require the use of ROX. 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 (T_m) 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). If you observe grouping at lower template concentrations, try adding more template.



5. REACTION SETUP

5.1 Gently vortex 2x MyGo Green 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 Green 1-Step Mix	10µl	1x	
Forward primer (10µM)	0.5µl	250nM	See 4.2 above
Reverse primer (10µM)	0.5µl	250nM	
20x RTase (contains RNase inhibitor)	1.0µl	1x	Add before template
Template	1pg to 1µg 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 SYBR® Green or FAM 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	