MyGo

REAGENTS FOR RESULTS

MyGo Green Mix Universal ROX

Cat. No. 6410 | 5935 | 8972 | 1841

Component	100 rxns (6410)	500 rxns (5935)	2000 rxns (8972)	5000 rxns (1841)
2x MyGo Green Mix No ROX	1 x 1ml	5 x 1ml	20 x 1ml	1 x 50ml
50µM ROX	1 x 150µl	1 x 150µl	4 x 150µl	1 x 520µl

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 Mix, 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 Green Mix provides highly accurate and sensitive quantification of DNA and cDNA targets in a convenient, easy-to-use format. The master mix includes MyGo HS Taq DNA Polymerase, dNTPs, and $MgCl_2$ in an optimised buffer to give the best results under challenging conditions such as high GC targets. The mix includes an intercalating dye which does not inhibit PCR, allowing DNA detection and analysis without the use of sequence specific probes.

MyGo HS Taq DNA Polymerase uses antibody-mediated hot start to provide highly specific and sensitive amplification. The enzyme is inactive at ambient temperatures, preventing the formation of primer-dimers and mis-primed products with the convenience of room temperature setup. The enzyme is activated at the start of a reaction with a 95°C incubation step.

ROX reference dye is provided separately for use with machines that recommend ROX. The MyGo range of instruments do not require ROX.

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. This kit contains ROX in case you want to use the kit with instruments that do require the use of ROX. Do not use the ROX additive in the MyGo instruments.

4.2 Addition of ROX

50µM ROX is supplied in a separate tube and is designed to be added directly to the 1ml tube of 2x MyGo master mix. Once ROX is added, the reagent may be used immediately or stored at -20°C for future use. Please check our ROX Selection Table at mygopcr.com to determine which ROX concentration your instrument requires. Use the tables below to add the correct amount of ROX and mix thoroughly after addition:

	Component	High ROX instruments	Final concentration	Reaction concentration
ROX for High ROX Instruments	2x MyGo Green Mix No ROX	1.0ml	2x	1x
	50µM ROX	20.0µl	1µM	500nM

	Component	Low ROX instruments	Final concentration	Reaction concentration
ROX for Low ROX Instruments	2x MyGo Green Mix No ROX	1.0ml	2x	1x
	50µM ROX	2.0µl	100nM	50nM

4.3 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.

5. REACTION SETUP

5.1 Gently vortex 2x MyGo Green Mix then prepare a master mix as follows:

Component	20µl reaction	Final concentration	Notes
2x MyGo Green Mix	10µl	1x	
Forward primer (10µM)	0.5µl	250nM	See 4.3 above
Reverse primer (10µM)	0.5µl	250nM	
Template DNA	<100ng cDNA <1µg gDNA	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	95°C	2 min	Polymerase activation: 2 minutes for cDNA, or 3 minutes for gDNA
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