MyGo

REAGENTS FOR RESULTS

# MyGo Probe Mix No ROX

Cat. No. 2003 | 2743 | 9809 | 3818

| Pack Size | Format | Presentation |
|---|---|---|
| 100 x 20µl rxns (2003) | 2x Ready Mix | 1 x 1ml |
| 500 x 20µl rxns (2743) | 2x Ready Mix | 5 x 1ml |
| 2000 x 20µl rxns (9809) | 2x Ready Mix | 20 x 1ml |
| 5000 x 20µl rxns (3818) | 2 x Ready Mix | 1 x 50ml |

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 Probe Mix provides fast, highly specific and ultra-sensitive real-time PCR and has been designed for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The master mix includes MyGo HS Taq DNA Polymerase, dNTPs, $MgCl_2$, stabilizers, and enhancers in an optimised buffer to give the best results under challenging conditions such as high GC and low-copy number targets.

MyGo Probe 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 enzyme is activated at the start of a reaction with a 95°C incubation step. The enhanced efficiency and specificity of MyGo Probe Mix make it the perfect choice for multiplex PCR.

MyGo Probe Mix No ROX is designed for use with real-time PCR instruments that do not require a passive reference.

## 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. For TaqMan® probes choose probe close to 5' primer and avoid terminal guanosine residues.

## 5. REACTION SETUP

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

| Component | 20µl reaction | Final concentration | Notes |
|---|---|---|---|
| 2x MyGo Probe Mix | 10µl | 1x | |
| Forward primer (10µM) | 0.5µl | 250nM | See 4.2 above |
| Reverse primer (10µM) | 0.5µl | 250nM | |
| Probe (10µM) | 0.4µl | 200nM | |
| Template DNA | <100ng cDNA<br><1µg gDNA | Variable | See 4.2 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 | 95°C | 2min | Polymerase activation: 2 minutes for cDNA or 3 minutes for gDNA |
| 40 | 95°C<br>60°C to 65°C | 10 seconds<br>20-30 seconds | Denaturation<br>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) |