



REAGENTS FOR RESULTS

## MyGo Green Mix High ROX

Cat. No. 3981 | 9242 | 2354 | 2569

| Pack Size               | Format        | Presentation |
|-------------------------|---------------|--------------|
| 100 x 20µl rxns (3981)  | 2x Ready Mix  | 1 x 1ml      |
| 500 x 20µl rxns (9242)  | 2x Ready Mix  | 5 x 1ml      |
| 2000 x 20µl rxns (2354) | 2x Ready Mix  | 20 x 1ml     |
| 5000 x 20µl rxns (2569) | 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](mailto:reagentsupport@mygopcr.com), providing full details including amplicon size, reaction setup, cycling conditions and screen grabs of amplification traces and melt profiles.

### 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<sub>2</sub> 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 included in the mix 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. Please check our ROX Selection Table at [mygopcr.com](http://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.

## 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 4.2 above |
| Reverse primer (10µM) | 0.5µl                    | 250nM               |               |
| 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 using the following conditions, 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<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  |