MyGo

REAGENTS FOR RESULTS

MyGo ProTaq Red Mix

Cat. No. 3416 | 2065

Component	80 Reactions (3416)	400 Reactions (2065)
2x MyGo ProTaq Red Mix	2 x 1ml	10 x 1ml

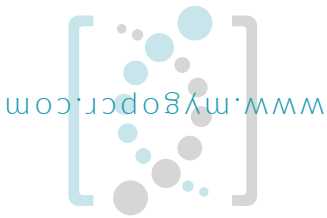
This product is for research use only

1. STORAGE

On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

2. TECHNICAL ASSISTANCE

If you have any questions, or experience any difficulties with MyGo ProTaq Red Mix, please email reagentsupport@mygopcr.com, providing full details including amplicon size, reaction setup, cycling conditions and screen shots of gel images.



3. DESCRIPTION

MyGo ProTaq Red Mix is a high-performance mix designed for the amplification of difficult templates. The mix includes MyGo ProTaq Polymerase, dNTPs, $MgCl_2$, and a red tracking dye in an optimised buffer designed to give the best results under challenging conditions such as the amplification of high GC targets and the presence of inhibitors. The tracking dye enables direct loading of PCR products onto agarose gels.

MyGo ProTaq Polymerase uses antibody-mediated hot start to give fast, highly specific PCR. 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. The enhanced efficiency and specificity of MyGo ProTaq Polymerase make it the ideal choice for the accurate amplification of long and complex templates.

MyGo ProTaq Red Mix is ready-to-use and supplied in a single tube containing all the reagents required for trouble-free PCR setup. It is recommended for the amplification of human genomic DNA up to 20kb. MyGo ProTaq Polymerase exhibits 2.5 fold higher fidelity than Taq DNA Polymerase. PCR products generated are A-tailed, and may be cloned into TA cloning vectors.

4. IMPORTANT NOTES

4.1 **2x MyGo ProTaq Red Mix:** The 2x mix contains MyGo ProTaq DNA Polymerase, 6mM $MgCl_2$, 2mM dNTPs, enhancers, stabilizers and a red tracking dye. The buffer system has been optimised for the best PCR results and we do not recommend adding further enhancers or $MgCl_2$ to the reaction.

4.2 **Template:** For cDNA use below 100ng per reaction. For eukaryotic DNA use between 5ng and 500ng per reaction.

4.3 **Primers:** 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. The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

4.4 **Annealing:** We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. As an alternative, you can use a 57°C annealing temperature then increase in 2°C increments if non-specific products are present.

4.5 **Extension:** The recommended extension temperature is 72°C. The optimal extension time will depend on amplicon length and complexity of template. For eukaryotic DNA we recommend 15 seconds per kilobase (kb) for amplicons below 5kb, and 40-60 seconds per kb for amplicons between 5-20kb.

4.6 **Dye migration:** MyGo ProTaq Red Mix includes a red dye for direct loading and tracking during agarose gel electrophoresis. The dye migration rate in a 2% agarose TAE gel is equivalent to 350bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 600bp of DNA.

5. REACTION SETUP

5.1 Prepare a master mix using the following table:

Component	50µl reaction	Final concentration	Notes
2x MyGo ProTaq Red Mix	25.0µl	1x	
Forward primer (10µM)	2.0µl	400nM	See 4.3 above
Reverse primer (10µM)	2.0µl	400nM	
Template DNA	<100ng cDNA <500ng gDNA	variable	See 4.2 above
PCR grade water	Up to 50µl total volume		

5.2 Cycle using the following conditions:

Cycles	Temperature	Time	Notes
1	95°C	1 min to 2 min	Initial denaturation and enzyme activation
25-35	95°C 55°C to 65°C 72°C	15 seconds 15 seconds 10 minutes*	Denaturation Anneal Extension (50 seconds per kb). *See 4.5 above