MyGo

REAGENTS FOR RESULTS

MyGo ProReader Polymerase

Cat. No. 8345 | 7893

Component	200 Units (8345)	1000 Units (7893)
MyGo ProReader Polymerase (2u/µl)	1 x 100µl	5 x 100µl
5x MyGo ProReader Buffer	3 x 1ml	15 x 1ml

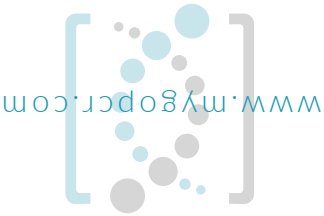
This product is for research use only

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 ProReader Polymerase, please email reagentsupport@mygopcr.com, providing full details including amplicon size, reaction setup, cycling conditions and screen shots of gel images.



3. DESCRIPTION

MyGo ProReader Polymerase is a robust and versatile high fidelity enzyme possessing 5'-3' polymerase and 3'-5' exonuclease proofreading activity. The enzyme is supplied with a 5x MyGo ProReader Buffer containing dNTPs and $MgCl_2$ optimised to give the best results under challenging conditions such as the amplification of high GC targets and the presence of inhibitors.

MyGo ProReader Polymerase is derived from Pfu DNA Polymerase and exhibits 50 fold higher fidelity than Taq DNA Polymerase, making it the ideal choice for applications where the DNA sequence needs to be correct after amplification.

The enzyme has an error rate of approximately 1 error per 4.5×10^7 nucleotides incorporated and will generate blunt-ended amplicons of up to 10kb in length.

4. IMPORTANT NOTES

4.1 **5x MyGo ProReader Buffer:** The 5x MyGo ProReader Buffer contains 15mM $MgCl_2$, 5mM dNTPs, enhancers and stabilizers. 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. We recommend 30 seconds per kilobase (kb) for amplification from eukaryotic gDNA or cDNA.

5. REACTION SETUP

5.1 Allow 5x MyGo ProReader Buffer to reach room temperature and gently vortex.

5.2 Prepare a master mix using the following table:

Component	50µl reaction	Final concentration	Notes
5x MyGo ProReader Buffer	10.0µl	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
MyGo ProReader Polymerase (2u/µl)	0.5µl		
PCR grade water	Up to 50µl total volume		

5.3 Cycle using the following conditions:

Cycles	Temperature	Time	Notes
1	95°C	1 minute	Initial denaturation
25-35	95°C 55°C to 65°C 72°C	15 seconds 15 seconds 30 seconds per kb	Denaturation Anneal Extension