COSMO PCR RED Master Mix (W1020300X)

Overview

This product contains *COSMO* Taq DNA Polymerase, isolated from a recombinant *E. coli* strain containing the DNA polymerase gene from *Thermus aquaticus* YT1 and is designed for high fidelity PCR.

Stages

1. Initial denaturation
2. Cycle denaturation
3. Annealing
4. Extension
5. Final extension

Features of *COSMO* PCR RED Master Mix

Accurate – the buffer provides the optimal pH and Mg^{2+} concentration to enhance enzyme performance and produce high yield and fidelity PCR products

Reliable – Willowfort kits undergo extensive tests to ensure consistent quality in all products

Time sensitive – Our DNA polymerase is designed to complete PCR in 30minutes

Product information

Catalog number	Package Size	No. of vials
WF10203001	100 reactions	2 x 1.25mL
WF10203002	200 reactions	4 x 1.25mL
WF10203003	1000 reactions	20 x 1.25mL

Required materials

- DNA template (10pg - 1µg)
- Specific gene Forward and Reverse primers
- Pipettes and tips
- Vortex
- 0.2mL PCR tubes
- Ice
- Nuclease Free Water
- Thermocycler
- Centrifuge

Reaction timings

Once each reaction tube has been set up, the PCR protocol will be complete in a minimum of 30 minutes. The amplified PCR product will be ready to combine with loading buffer and run on an agarose gel for analysis.

Storage conditions

The reagents provided in the *COSMO* PCR Master Mix should be stored at -20^{0}C. Where possible stored reagents in conditions of non-frost-free.

Warning!

Please be advised to wear appropriate eyewear, clothing and gloves. Read the Safety Data Sheets (SDS) for any further safety information

Any further information

For further information and protocols, visit our website https://willowfort.co.uk/. For any additional support, please contact us through https://willowfort.co.uk/contact-us

For research use only. These products should not be used for diagnostics

Components of *COSMO* PCR Master Mix

COSMO PCR Master Mix

- ***COSMO* Taq DNA Polymerase –** a thermostable DNA polymerase used for DNA synthesis. Stored in buffer to prevent degradation.
- ***COSMO* PCR RED buffer** contains the optimal concentrations of $(NH_4)_2SO_4$ and KCl. In addition, it is buffered to pH9 to maximise performance.
  - **$MgCl_2$** – This is included within the buffer since the polymerase requires Mg^{2+} as a cofactor to function. If the DNA template has been extracted with chelating agents such as EDTA, additional Mg^{2+} ions are required to maintain enzyme performance. We recommend adding 5µl of 25mM $MgCl_2$ *(not included).*
  - **dNTPs** – This is included in the *COSMO buffer.* The purity of dNTPs in all Willowfort products is ≥99%, to ensure maximum fidelity and speed. For longer sequences, additional dNTPs maybe required *(not included, but additional vials can be purchased on our website).*
  - **Storage buffer –** This is included in the buffer. 20mM Tris buffer pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT and glycerol to prevent enzyme degradation.

Protocol for *COSMO* PCR RED Master Mix

1. Thaw all reagents and store on ice. Mix and centrifuge all reagents well before preparing the master mix. Check for precipitation
2. Combine all reagents together in a nuclease free eppendorf, using Table 1 as a guide. Mix thoroughly and briefly centrifuge.

Table 1: Reagents required per sample

Reagents	Volume
COSMO PCR RED Master Mix	25µl
Forward and Reverse Primers (20µM)	1—2.5µl (0.1—1µM each)
Nuclease free water	To 50µl
DNA template (10pg—1µg)	Volume varies

3. Aliquot the master mix into separate 0.2mL PCR tubes before adding the DNA template.

Table 2: Temperature and Times for the PCR protocol

Stage	Temperature	Time
Stage 1	95°C	*2 min
Stage 2.1	95°C	15 sec
Stage 2.2	Primer Tm <5°C	20 sec
Stage 2.3	72°C	#30-60s
Repeat stage 2 for 25-35 cycles		
Stage 3	72°C	1 min
*Increase to 5min if GC content is higher than ≤50% # The extension time depends upon the amplicon length		

4. Gently mix the components together and centrifuge briefly.
5. Use Table 2 to set up the thermal profile, considering the melting temperature of the primers.
6. Add and incubate samples in the thermocycler
7. Once complete, PCR products can be combined with gel loading buffer and loaded into an agarose gel. The PCR products can be analysed after electrophoresis

Additional protocol information

Deactivating agents – This can be achieved by liquid-liquid extraction (phenol or chloroform), or by ionic detergent extraction.

Tm calculation – For primers bellow 25 nucleotides, please use the equation bellow to calculate the Tm:

$$Tm = 4(G+C) + 2(A+T)$$

If the primer has more than 25 nucleotides this equation is insufficient, and computational methods are required.

Template – For plasmids and phage DNA use 0.01-1ng and for genomic DNA use 0.1-1µg for optimal accuracy.

Troubleshooting – For any troubleshooting queries, please visit our website https://willowfort.co.uk/end-point-pcr/cosmo-pcr-master-mix. Any further information, please contact us, via https://willowfort.co.uk/contact-us.