Microzone Product Datasheet

Product code: 2MMCR- Lot Number:

2X MegaMix Crystal Q5 High Fidelity Red Mastermix

P. Code	Size in 100 Rxn	Size in 500 Rxn	Component	Description	Lot Number	Expiry
2MMCR-1.25	1 x 1.25 mL	5 x 1.25 mL	2X MegaMix Crystal Q5 High Fidelity Red Mastermix	Specially optimised reaction buffer containing Q5 HotStart High Fidelity Polymerase, dNTPs, $MgSO_4$, enhancers and red loading dye.		
MFO-1	1 x 1 mL	5 x 1 mL	4X microFORCE	Buffer solution optimised for templates with high GC content or GC-repeats.		
5JWA-1	1 x 1 mL	5 x 1 mL	Just Water (Molecular Grade Water)	Aliquoted, quality controlled, nuclease-free, molecular grade water.		

Applications

- Long-range PCR amplifications (up to 40 kb)
- NGS library preparation and Sequencing
- Site-directed Mutagenesis
- Amplification of difficult (GC-rich) templates

Product Description

2X MegaMix Crystal Q5 High Fidelity Red Mastermix

The 2X MegaMix Crystal Q5 High Fidelity Red Mastermix consists of Q5 DNA Polymerase in an enhanced buffer with optimal Mg^{2+} concentration, balanced dNTPs, a proprietary formulation of stabilisers and enhancers specifically optimised for our enzyme and an inert red loading dye. This convenient 2X mastermix eliminates uncertainties in PCR, ensuring reproducible results.

Q5 High Fidelity DNA Polymerase offers unmatched accuracy with a 280-fold higher fidelity compared to Taq Polymerase and a 5-fold higher fidelity compared to Pfu Polymerase. This enzyme features a Hot-Start mechanism for increased specificity and sensitivity, allowing for room temperature reaction setup. Expertly engineered, Q5 DNA Polymerase can efficiently generate products up to 40 kb, with faster extension times than many other high-fidelity polymerases, making it ideal for long and complex targets.

The red agarose loading dye incorporated allows easy visualisation and eliminates the need for additional gel loading buffers. The dye does not inhibit restriction enzymes or ligases and does not fluoresce at the wavelengths used by automated DNA sequencers so downstream processes are not impacted. In a 1% gel it migrates at around the same speed as 500 bp fragment.

It is the ideal enzyme for cloning and various sequencing applications, including NGS and Sanger. The PCR products produced are blunt-ended and can be ligated into blunt vectors.

4X microFORCE

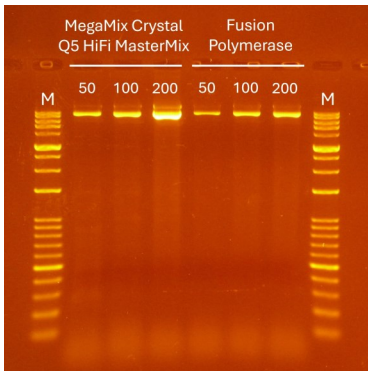
Microzone's enhancing buffer for the amplification of difficult templates.

Just Water

Our molecular biology grade water, providing high purity with the complete absence of DNase and RNase.

Key Features

- Highest fidelity amplification (~280X higher than Taq)
- Suitable for amplicons up to 40 kb
- Ultra-low error rate due to 3´-5´ exonuclease proofreading activity
- Ideal for cloning, sequencing and long or difficult amplicons
- Hot-Start version for increased specificity and sensitivity



Superior amplicon yield using MegaMix Crystal Q5 High Fidelity MasterMix vs commercially available Fusion polymerases. Gel image shows amplification of 8.5 kb fragment from human gDNA, using template concentrations of 50, 100 and 200 ng. PCR was performed in 50 µL reaction using Microzone's MegaMix Crystal Q5 High-Fidelity range. M: 1 kb DNA Ladder.

Protocol

Thaw all reagents completely and mix well before use.

Components	Volume
2X MegaMix Crystal Q5 HiFi Red MasterMix	25 µL
4X microFORCE (optional: for GC-rich templates only)	12.5 µL
Primers	x µL
Template	y µL
Just Water (Molecular Grade Water)	make up to 50 µL

Prepare a master mix as described in the table below.

Mix gently, avoiding bubbles, centrifuge if necessary.

Include a no template control and positive control as required.

Thermocycling

The following general cycling conditions are intended for use as a guide and can vary depending on the template and primers being used.

For the amplification of long fragments contact Microzone for recommendations.

Step	Cycles	Temperature	Time
Initial Denaturation	1	98°C	30 seconds
Denaturation		98°C	5-10 seconds
Annealing	25 - 35	60 - 68°C	10-15 seconds
Elongation		72°C	30 seconds / kb
Final Elongation	1	72°C	2 min

For Research Use Only. Not for use in diagnostic procedures.

Product Handling and Storage

To ensure the quality of the product until the expiry date keep at the recommended storage temperature and limit exposure to light.

Licenses

This product is covered by one or more patents.

This product is licensed for research and commercial use from Bio-Rad Laboratories, Inc., under U.S. Pat. Nos. 6,627,424, 7,541,170, 7,670,808, 7,666,645, and corresponding patents in other countries. No rights are granted for use of the product for Digital PCR or real-time PCR applications, with the exception of quantification in Next Generation Sequencing workflows.

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