High Fidelity Phusion

PCR Master Mix 2X

www.genetoprotein.com | quote@genetoprotein.com

Product Information

Catalogue numbers: PCR007 | PCR008
Sample packs: SP-PCR007
Format: Ready-to-use 2X PCR master mix, without dye
Use: High-fidelity endpoint PCR and genotyping

Storage & Handling

Store at −20°C; minimize repeated freeze–thaw cycles.
Thaw on ice, then mix gently by inversion. Do not vortex.
Keep reactions on ice during setup; loading dye is separate.

Principle and Applications

G2P High Fidelity Phusion PCR Master Mix 2X is a ready-to-use amplification mixture containing a high-fidelity DNA polymerase, dNTPs, $MgCl_2$ and reaction-buffer components. Combined at 1X with DNA template, forward and reverse primers, and nuclease-free water, it supports efficient amplification with fewer pipetting steps. The high-fidelity formulation is suited to genotyping, PCR product analysis and workflows requiring accurate DNA synthesis. The master mix does not contain DNA loading dye; the separate 6X dye is used after amplification for agarose-gel analysis. We also have the version with PCR Mix with dye PCR005/ PCR006.

Ordering Information and Kit Contents

Component / pack	PCR007	PCR008	Sample packs
Catalogue number	PCR007	PCR008	SP-PCR007
Pack size	1.25 mL	4 × 1.25 mL	125 µL
High Fidelity Phusion Master Mix 2X	1.25 mL	4 × 1.25 mL	125 µL
50 mM $MgCl_2$ (supplement)	100 µL	400 µL	See pack label

Standard PCR Reaction Setup

Prepare reactions on ice using nuclease-free PCR tubes. The table gives a starting composition for 25 µL and 50 µL reactions. Adjust DNA input and primer conditions for the target and template.

Component	25 µL reaction	50 µL reaction	Final concentration
DNA template	Variable	Variable	Template dependent
Forward primer (10 µM)	0.5 µL	1.0 µL	0.2 µM
Reverse primer (10 µM)	0.5 µL	1.0 µL	0.2 µM
2X PCR Master Mix	12.5 µL	25 µL	1X
Optional $MgCl_2$ stock	As required	As required	Only after optimization
Nuclease-free water	To 25 µL	To 50 µL	Final reaction volume

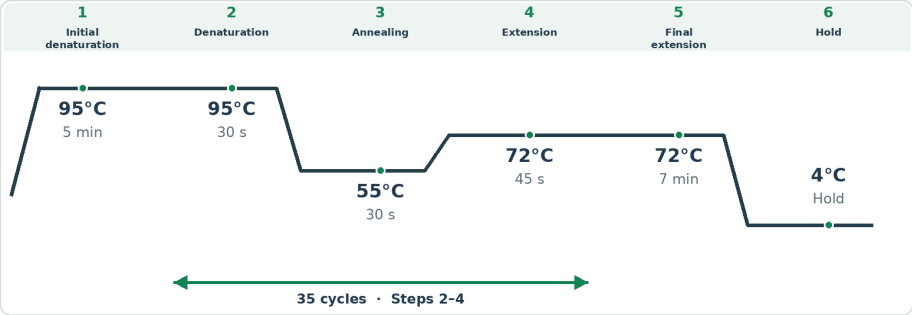
Handling note. Mix the master mix gently after thawing; do not vortex. Because the 2X mix already contains magnesium and dNTPs, additional $MgCl_2$ is normally unnecessary. Use the separately supplied loading dye only when preparing the PCR products for electrophoresis.

Recommended Thermal Cycling Programme

Example cycling conditions for an approximately 1 kb DNA target. Annealing temperature should be selected based on primer melting temperature; extension time may require adjustment for longer amplicons.

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	5 min	1 cycle
Denaturation	95°C	30 s	35 cycles
Annealing	55°C	30 s	
Extension	72°C	45 s	
Final extension	72°C	7 min	1 cycle
Hold	4°C	Until recovery	—

Figure 1. Standard thermal-cycling programme for endpoint PCR.



Cycling guidance. The illustrated 55°C annealing temperature is a starting point, not a universal setting. Optimize for primer Tm and template. For longer fragments, use approximately 1 min/kb at 72°C as an initial extension-time guide.

Representative Amplification Result

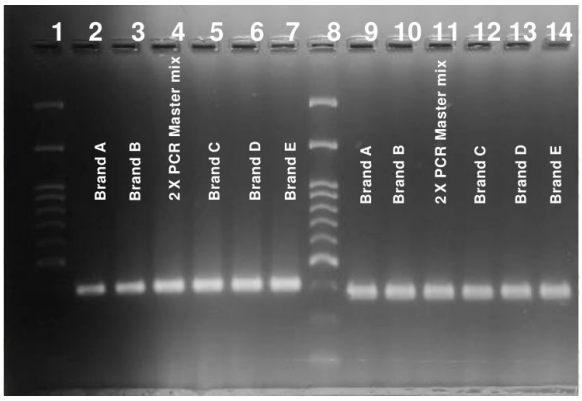


Figure 2 | Plant genotyping PCR

Representative agarose-gel electrophoresis of PCR products amplified from plant genomic DNA using G2P High Fidelity Phusion PCR Master Mix 2X and comparator PCR master mixes.

Lanes 1 and 8: DNA ladders. Lanes 4 and 11: G2P Master Mix 2X. Remaining lanes: comparator master mixes A–E, as labeled in the image.

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Reaction Component Guidance

Component	Practical guidance
DNA template	Use clean DNA at an appropriate concentration. Purification carryover, excess template or degraded DNA can reduce PCR yield.
Primers	Begin near 0.2 µM of each primer; choose the annealing temperature using primer Tm and target specificity.
Master Mix	Add 12.5 µL of the supplied 2X master mix per 25 µL PCR to achieve a final 1X mixture. Avoid repeated freeze–thaw cycles.
$MgCl_2$ and loading dye	Additional 50 mM $MgCl_2$ is for optimization only; the 6X loading dye (not included) is used after PCR, not during cycling.

Troubleshooting Guide

If amplification is weak, absent or nonspecific, examine template integrity, primer design, master-mix handling and thermal-cycling conditions. Include a positive control and a no-template control when troubleshooting.

Observation	Possible cause	Recommended action
No or low PCR product	Low-quality or insufficient template	Verify DNA concentration and integrity; dilute or clean up potentially inhibitory samples.
No or low PCR product	Unsuitable primer annealing temperature	Check primers and use a temperature-gradient PCR to optimize annealing.
No or low PCR product	Omitted component or short extension	Confirm the 2X master mix and primers were added; increase extension time for longer products.
Multiple bands	Annealing temperature too low	Increase annealing temperature or improve primer specificity.
Smearing	Excess template or inhibitory carryover	Reduce template input and assess salt or purification-reagent contamination.
Primer-dimer	Primer complementarity	Review primer sequences; titrate primer concentration.
Weak GC-rich amplification	Difficult secondary structure	Use a validated GC-rich PCR strategy and optimize denaturation/annealing conditions.
Positive no-template control	Contaminated reagents or work area	Replace suspect reagents; use filtered tips and physically separate pre- and post-PCR work.
Inconsistent results	Poor mixing or multiple freeze–thaw cycles	Aliquot the mix, thaw on ice and mix by gentle inversion. Do not vortex.

Additional Information

Principle. This 2X mix contains the amplification reagents needed in a single master mix, apart from primers, DNA template and nuclease-free water. High-fidelity PCR products are generally blunt-ended; A-tailing may be required for conventional TA cloning.

Storage. Keep at −20°C and minimize freeze–thaw cycles. Thaw on ice and mix by gentle inversion. Use sterile nuclease-free consumables.

Separate reagents. $MgCl_2$ supplementation is optional for assay optimization. The 6X DNA Loading Dye is supplied separately for post-PCR gel analysis and is not part of the 2X amplification mixture.

Applications. Suitable for routine high-fidelity PCR, DNA fragment amplification and plant genotyping workflows. For Research Use Only.

Ordering. PCR007: 1.25 mL; PCR008: 4 × 1.25 mL; SP-PCR007: 125 µL sample packs.