G2P Standard PCR

Master Mix 2X

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Product Information
Catalogue numbers: PCR011 | PCR012
Sample pack: SP-PCR011 (125 µL)
Format: Ready-to-use 2X PCR mix, without loading dye
Use: Routine endpoint PCR amplification

Storage & Handling
Store at −20°C; avoid repeated freeze–thaw cycles.
Thaw on ice and mix gently by inversion.
Do not vortex. Loading dye is supplied separately with full-size kits.

Principle and Applications

Standard PCR Master Mix 2X (without dye) contains all the components required for a successful polymerase chain reaction, which includes appropriate amounts of dH2O, DNA polymerase, dNTPs, MgCl2, and reaction buffers for effective amplification. This master mix formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible PCR. The master mix contains polymerase which is a high-fidelity polymerase. In most cases, the error rate is 1 in every 1.3 million base pairs. At 72°C, the DNA Polymerase has a processivity of roughly 1 kb/min. It is compatible with TA cloning

Ordering Information and Kit Contents

Component / pack	PCR011	PCR012	Sample pack
Catalogue number	PCR011	PCR012	SP-PCR011
Pack size	1.25 mL	4 × 1.25 mL	125 µL
Standard PCR Master Mix 2X	1.25 mL	4 × 1.25 mL	125 µL
Nominal 25 µL PCR reactions	100	400	10
50 mM $MgCl_2$ (separate)	100 µL	400 µL	—*
6X DNA loading dye (separate)	600 µL	2.4 mL	—*

* The supplied sample-pack update specifies 125 µL of master mix (10 reactions); auxiliary sample-pack components were not stated. No sample pack is available for PCR012.

Standard PCR Reaction Setup

Prepare reactions on ice with nuclease-free tubes and filtered tips. The volumes below produce a final 1X master-mix concentration.

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 Standard PCR Master Mix	12.5 µL	25 µL	1X
Nuclease-free water	To 25 µL	To 50 µL	Final reaction volume

Handling note. Thaw mix on ice, mix by gentle inversion, and briefly spin down. Add 6X DNA loading dye separately only after PCR when preparing an aliquot for agarose-gel analysis.

PCR-to-Gel Workflow

1 Set up PCR

Combine 2X master mix, primers, template and water.

2 Amplify

Run the thermal-cycling programme and optimize as needed.

3 Prepare gel sample

Add separate loading dye; resolve PCR products on an agarose gel.

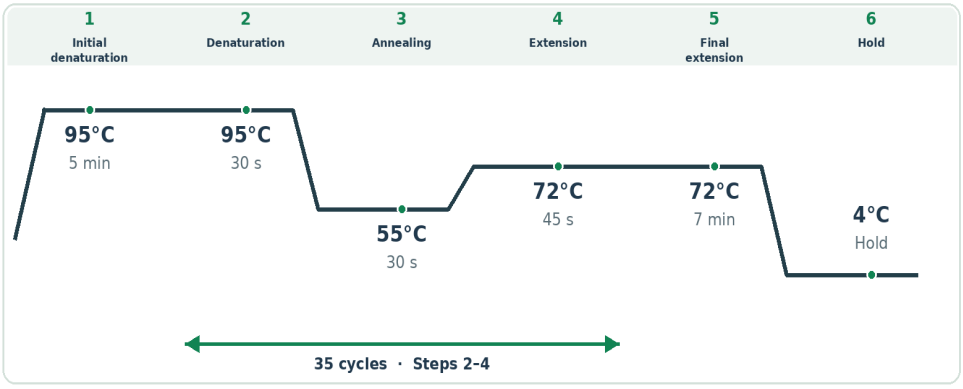
Gel visualization: A suitable DNA gel stain and electrophoresis buffer are required; loading dye does not stain DNA.

Recommended Thermal Cycling Programme

Suggested starting programme for a DNA target of approximately 1 kb. Annealing temperature and extension duration should be adjusted for primer Tm, target length and template characteristics.

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	5 min	1 cycle
Denaturation	95°C	30 s	
Annealing	55°C	30 s	35 cycles (steps 2–4)
Extension	72°C	45 s	
Final extension	72°C	7 min	1 cycle
Hold	4°C	Until recovery	—

Figure 1. Suggested standard thermal-cycling programme for endpoint PCR.



Cycling guidance. The 55°C annealing setting is an example, not a universal condition. Adjust according to primer melting temperatures. For longer products, approximately 1 min/kb at 72°C may be used as an initial extension-time guide.

Representative Amplification Result

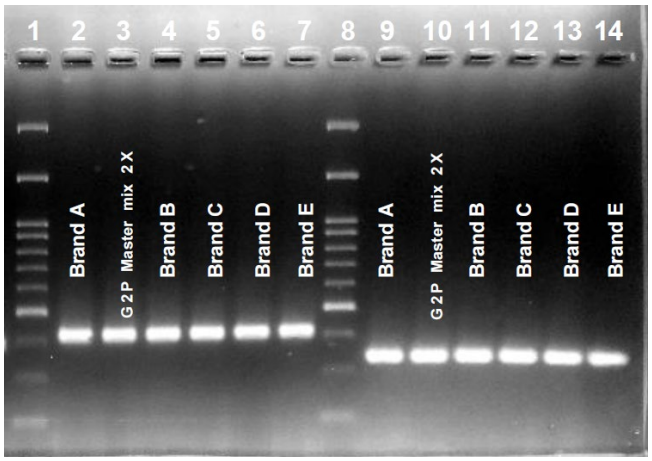


Figure 2 | Representative PCR amplification

Agarose-gel image reproduced from the previous G2P Standard PCR Master Mix 2X datasheet. The original figure shows DNA amplification using the G2P mix and comparison mixes.

Reaction Component Guidance

Component	Practical guidance
DNA template	Use purified DNA at a suitable concentration; poor quality or excess template can inhibit amplification.
Primers	Start at 0.2 µM each; optimize the annealing temperature for the primer Tm and target specificity.
2X master mix	Use 12.5 µL in a 25 µL reaction, or 25 µL in a 50 µL reaction, to obtain a final 1X concentration.
$MgCl_2$ supplement	The original full-size kits have a separate 50 mM $MgCl_2$ solution; adjust only when optimizing an assay.
Post-PCR gel loading	The 2X mix contains no loading dye. Add separate 6X dye before electrophoresis; use an appropriate DNA stain.

Troubleshooting Guide

When amplification is absent, weak or nonspecific, review template integrity, primer design, pipetting accuracy, reagent handling and cycling conditions. Run a positive control and a no-template control where appropriate.

Observation	Possible cause	Recommended action
No or low PCR product	Low-quality or insufficient template	Check DNA quality and quantity; dilute or repurify samples containing inhibitors.
No or low PCR product	Unsuitable primer annealing temperature	Check primer Tm and optimize with a gradient PCR.
No or low PCR product	Omitted component or inadequate extension	Verify that the mix, primers and DNA were added; extend the 72°C step if needed.
Multiple or nonspecific bands	Low annealing stringency	Increase or optimize annealing temperature; review primer specificity.
Smearing	Excess template or carryover contaminants	Reduce template input and assess salt or extraction-reagent carryover.

Primer-dimer	Primer complementarity or excessive primer	Review primer sequences and consider reducing primer concentration.
Weak GC-rich amplification	Stable secondary structures	Optimize annealing/denaturation; use a validated GC-rich assay strategy.
Positive no-template control	PCR contamination	Replace contaminated reagents; separate pre- and post-PCR work areas.
Inconsistent results	Poor mixing or repeated freeze–thaw	Aliquot as needed, thaw on ice, and mix gently by inversion; do not vortex.

Additional Information

Formulation. The 2X master mix contains DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer. Prepare reactions at a final 1X mix concentration with the intended DNA template and primer pair.

Storage. Store at −20°C. Avoid unnecessary freeze–thaw cycles; prepare single-use aliquots if repeated use is expected. Thaw on ice and mix gently without vortexing.

Post-PCR analysis. The master mix is supplied without premixed loading dye. Separate 6X DNA loading dye is needed to load on Agarose gel. Combine one volume of 6X dye with five volumes of completed PCR sample before loading,

Applications. Suitable for routine endpoint PCR, screening and genotyping workflows, subject to assay-specific optimization.

Technical note. The cycling conditions provided are starting recommendations, not validated settings for every primer or template. Confirm performance under the intended experimental conditions.