For life science research only.
Not for use in diagnostic procedures.

High Fidelity PCR Master

Version: 12
Content Version: November 2021

Ready-to-use reaction mix for convenient and reliable amplification of specific DNA fragments by PCR.

Cat. No. 12 140 314 001 1 kit
200 reactions in a final volume of 50 µl

Store the mix +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	High Fidelity PCR Master, High Fidelity PCR Master	▪ Ready-to-use solution. ▪ Contains enzyme blend, double-concentrated reaction buffer with 3 mM $MgCl_2$, and 0.4 mM each dATP, dCTP, dGTP, dTTP. ▪ Each vial is for 20 reactions.	10 vials, 500 µl each
2	High Fidelity PCR Master, Water, PCR Grade	To adjust final reaction volume.	5 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the mix is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	High Fidelity PCR Master	Store at +2 to +8°C. For long-term storage, store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	Water, PCR Grade	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Autoclaved reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as 0.2 ml thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

For standard PCR

- PCR primers
- Template DNA

1.4. Application

Use the High Fidelity PCR Master instead of the Expand High Fidelity PCR System or instead of single reagents for nearly all PCR applications.

⚠ ***The only limitation is that the sample volume must not exceed half the total reaction volume.***

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use template DNA, such as genomic or episomal, or plasmid DNA suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids, use:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, and PCR primers vary from system to system and must be determined for each individual experimental system.

⚠ ***Perform the elongation step at +68°C when PCR products longer than 3 kb are amplified. Be aware that the 3′ → 5′ exonuclease activity will repair mismatched primers at the 3′ end.***

Prevention of Carryover Contamination

dUTP is a poor substrate for the High Fidelity PCR Master.

⚠ ***Do not use it in combination with Uracil-DNA Glycosylase for carryover prevention.***

2.2. Protocols

Preparation of PCR master mix

The preparation of a master mix circumvents the need for hot start and avoids that the enzyme blend interacts with primers or template which could lead to a partial degradation of primer and template by the 3′ → 5′ exonuclease activity of Tgo DNA Polymerase.

1. To an autoclaved 1.5 ml reaction tube on ice, add the components in the order listed for each 50 µl reaction.

Reagent	Volume [µl]	Final conc.
Forward primer	variable	300 nM
Reverse primer	variable	300 nM
Template DNA	variable	10 – 500 ng (up to 500 ng genomic DNA, 10 ng plasmid DNA)
Water, PCR Grade	add up to 25	–
Total Volume	**25**	

2. Mix and centrifuge briefly to collect the sample at the bottom of the tube.

3. Pipette the PCR master mix with 25 µl High Fidelity PCR Master into thin-walled PCR tubes on ice and mix well.

PCR protocol

The following thermal profiles are an example. Different thermal cyclers may require different profiles.

1. Place your samples in a thermal block cycler and use the thermal profiles below to perform PCR.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation Annealing Elongation	94 45 – 65[1] 68 or 72[2]	10 sec 70 sec 45 sec – 4 min[3]	10
Denaturation Annealing Elongation	94 45 – 65[1] 68 – 72[2]	15 sec 30 sec 45 sec – 4 min[3] + 5 sec cycle elongation for each successive cycle	15 – 20
Final Elongation	72	up to 7 min	1

2. After cycling, use samples immediately or store at −15 to −25°C for later use.

For best results, check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker. In addition, purify the PCR product with the High Pure PCR Product Purification Kit, for example, before performing nested PCR.*

[1] Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.
[2] Elongation temperature depends on the length of the amplification product. For PCR products up to 3 kb, elongation temperature should be +72°C; for PCR products >3 kb, elongation temperature should be +68°C.
[3] Elongation time depends on fragment length. For example, use 1 minute for a 1.5 kb fragment, 4 minutes for 5 kb fragment, etc. Be sure to use the cycle extension features.

2.3. Parameters

Error Rate

Approximately three-fold increased fidelity of DNA synthesis compared to Taq DNA polymerase.

Incorporation of Modified Nucleotides

High Fidelity PCR Master accepts modified nucleotides, such as DIG-11- dUTP*, Biotin-16-dUTP*, and Fluorescein-12-dUTP*. Labeling with DIG-dUTP is achieved by adding the modified nucleotide to a final concentration of 10 µM.

Maximum Fragment Size

Up to 5 kb.

PCR Cloning

TA and blunt-end cloning.
Generates a mixture of PCR products with blunt ends and 3′-single A overhangs.

3. Results

Amplification of different targets from human genomic DNA

The ability of the High Fidelity PCR Master to amplify different targets without individual adjustment of the reagent compositions is demonstrated (Figure 1). The result shows that fragments ranging from 1.1 kb up to 4.8 kb can be obtained in a high yield and high specificity.

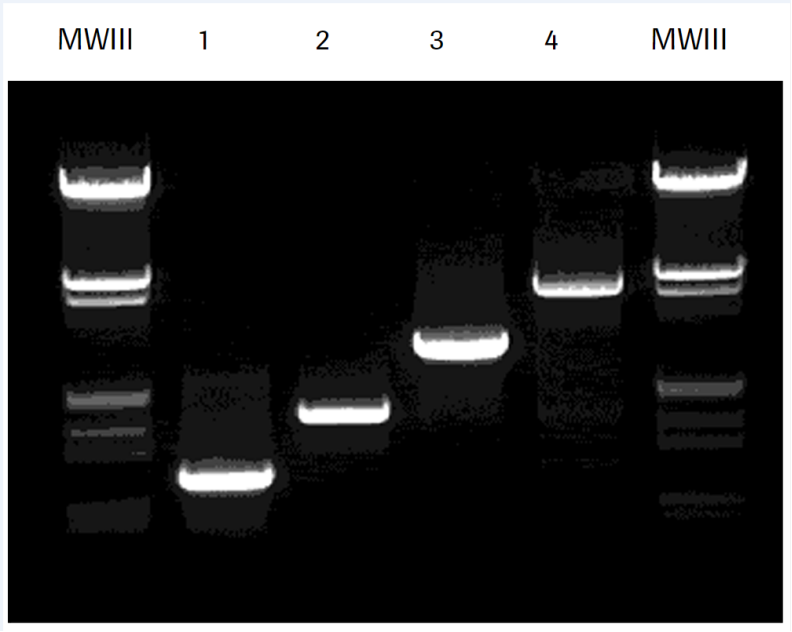

Fig. 1: Fragments of different lengths of human genomic DNA were amplified under standard conditions.
Lane 1: High Fidelity PCR Master, 1.1 kb Collagen fragment
Lane 2: High Fidelity PCR Master, 1.7 kb tPA fragment
Lane 3: High Fidelity PCR Master, 2.9 kb p53 fragment
Lane 4: High Fidelity PCR Master, 4.8 kb tPA fragment
MWIII: Molecular weight marker III

4. Troubleshooting

Observation	Possible cause	Recommendation
No PCR product.	Pipetting errors or omission of a reagent.	Repeat PCR with the same sample and PCR reagents.
	Unbalanced reaction	Check final concentrations of your components.
	Cycle conditions not optimal.	Check cycle conditions.
	Template concentration too low.	Increase concentration of template.
	Template quality poor (degraded, contaminated, contains inhibitors).	Use primers that amplify smaller genomic sequences.
		Prepare new template with the High Pure PCR Template Preparation Kit* which produces high molecular weight, pure DNA.
		Store template at +2 to +8°C. ⚠ ***For long-term storage, the template may be stored at −15 to −25°C, however, avoid repeated freeze/thaw cycles.***
	Primer concentration or sequence not optimal.	Optimize primer concentration.
		Verify primer sequences.
		Design alternative primers.
	Multiple contributing factors.	Test reaction with a positive control template and primers of known performance.
		Start over; use freshly made solutions of master mix, template, and primers.
	High Fidelity PCR Master not performing adequately.	Check High Fidelity PCR Master performance with a positive control template and primers.
		Use a new batch of High Fidelity PCR Master.
		Increase cycle number.
Product is smeared.	Secondary amplification product.	Check concentration and cycle conditions.
		Optimize/check primer concentration.
		Decrease number of cycles.
		Check and if necessary, decrease concentration of template.
Nonspecific product bands present.	Nonspecific binding of primers.	Use higher annealing temperatures to get the highest specificity.
		Check and optimize primer concentration.
		Redesign primers for more specific binding of target and/or to allow a higher annealing temperature.

5. Additional Information on this Product

5.1. Test Principle

The High Fidelity PCR Master is composed of a unique enzyme mix containing two thermostable polymerases: Taq DNA Polymerase and Tgo DNA Polymerase, a thermostable polymerase with proofreading activity.

- This powerful specific enzyme blend is designed to give PCR products with high yield, high fidelity, and high specificity from episomal and genomic DNA.
- Due to the inherent 3′-5′ exonuclease activity of the proofreading polymerase, the High Fidelity PCR Master results in approximately three times the fidelity and approximately twice the yield of Taq DNA Polymerase alone.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910
Digoxigenin-11-dUTP, alkali-stable	25 nmol, 25 µl, 1 mM	11 093 088 910
	125 nmol, 125 µl, 1 mM	11 558 706 910
	5 x 125 nmol, 5x 125 µl, 1 mM	11 570 013 910
Biotin-16-dUTP	50 nmol, 50 µl, 1 mM	11 093 070 910
Fluorescein-12-dUTP	25 nmol, 25 µl, 1 mM	11 373 242 910
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
High Pure PCR Template Preparation Kit	1 kit, up to 100 purifications	11 796 828 001

6.4. Trademarks

MAGNA PURE and EXPAND are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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