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

FastStart PCR Master

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2x-concentrated, ready-to-use hot start master mix for PCR on thermal block cycler instruments.

Cat. No. 04 710 436 001	1 kit 2 x 1.25 ml 100 reactions in a final volume of 50 µl
Cat. No. 04 710 444 001	1 kit 8 x 1.25 ml 400 reactions in a final volume of 50 µl
Cat. No. 04 710 452 001	1 kit 10 x 5 ml 2,000 reactions in a final volume of 50 µl

Store the mix at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	FastStart PCR Master, 2x conc.	▪ Ready-to-use hot start PCR mix. ▪ Contains FastStart Taq DNA Polymerase, double-concentrated reaction buffer, 0.4 mM each dATP, dCTP, dGTP, dTTP, and $MgCl_2$.	04 710 436 001	2 vials, 1.25 ml each
			04 710 444 001	8 vials, 1.25 ml each
			04 710 452 001	10 vials, 5 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the mix is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	FastStart PCR Master, 2x conc.	Store at −15 to −25°C. For short-term storage up to 1 month, store at +2 to +8°C. ⚠ ***Avoid repeated freezing and thawing.***

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
- Water, PCR Grade*

For the reverse transcription (optional for RT-PCR)

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*

For DNA labeling with modified dNTPs

- Digoxigenin-11-dUTP, alkali-stabile*, or
- Digoxigenin-11-dUTP, alkali-labile*
- Biotin-16-dUTP*
- Fluorescein-12-dUTP*

1.4. Application

The FastStart PCR Master offers a convenient solution for PCR amplifications not requiring individual adjustment of the reagent compositions. The master mix can be used for:

- All hot start applications.
- Amplification of genomic DNA and cDNA targets up to 2 kb long with high specificity, sensitivity, and yield.
  Longer fragments are possible on plasmid DNA targets.
- Simple Multiplex PCR.
- Automated PCR which requires handling at +15 to +25°C.

The mix is designed for optimal amplification of targets up to 2 kb long from complex templates, such as genomic DNA. Do not use the mix to amplify longer targets. The only limitation is that the sample and primer volume must not exceed half the total reaction volume.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as genomic or plasmid DNA, cDNA 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).
- Use up to 500 ng complex genomic DNA or 100 ng plasmid DNA/cDNA.

Store the template DNA in either Water, PCR Grade or 5 to 10 mM Tris-HCl, pH 7 to 8. Avoid dissolving the template in TE buffer since EDTA chelates Mg^{2+}.

Control Reactions

Negative control

To detect possible DNA contamination, always include a negative control in each run. To prepare this control, replace template DNA with Water, PCR Grade*.

Primers

0.1 to 0.4 µM each (final concentration)
0.3 µM each (standard concentration).

Always use equimolar primer concentrations.

The design of the PCR primers determines amplicon length, melting temperature, amplification efficiency, and yield. Several programs for primer design are freely available to the public on the Internet.

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.

Reaction volume

Various reaction volumes of the FastStart PCR Master can be used. Refer to recommendations from the supplier of the thermal block cycler instrument for suitable volumes and tubes/plates.

2.2. Protocols

Preparation of PCR mix

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.
   – Mix solutions carefully by pipetting them up and down, then store on ice.

2. Prepare a 10x-concentrated solution of each respective primer.

   If you are using, for example, the final concentration of 0.3 µM for each primer, the 10x-concentrated solution would contain a 3 µM concentration of the respective primer.

3. To an autoclaved 1.5 ml reaction tube on ice, add the components in the order listed for each 50 µl reaction.
   – To prepare the PCR mix for more than one reaction, multiply the amount in the column “Volume” by the number of reactions plus sufficient additional reactions.

Reagent	Volume [µl]	Final conc.
FastStart PCR Master, 2x conc.	25	1x
Forward primer 1 (3 µM)	5	300 nM
Reverse primer 2 (3 µM)	5	300 nM
Water, PCR Grade*	10	–
Total Volume	**45**	

4. Mix solution carefully by pipetting it up and down.

   ⚠ ***Do not vortex.***

   - Pipette 45 µl PCR mix into thin-walled PCR tubes or well of a PCR microplate, depending on your block cycler instrument.

5. Add 5 µl template DNA (up to 250 ng genomic DNA or 100 ng plasmid DNA/cDNA).

   In initial experiments, to determine the optimum amount of cDNA template, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel. Too much DNA may inhibit the PCR.

PCR protocol

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

1. Prepare tubes or microplates for PCR according the the instrument supplier.

2. 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/activation	95[1]	4 min	1
Denaturation	95	30 sec	30 – 40[2]
Annealing	45 – 65[3]	30 sec	
Elongation	72	45 sec – 3 min[4]	
Final Elongation	72	7 min	1

[1] This step activates the previously inactive FastStart Taq DNA Polymerase and denatures the DNA template. Yield of PCR product might be increased by longer activation time up to 6 minutes or more cycles. Activation times down to 2 minutes will give good results. Yield and specificity in a multiplexing-PCR might be increased by longer activation time up to 10 minutes or more cycles.

[2] 30 cycles are enough to produce an adequate amount of product, if there is sufficient target (preferably $>10^4$ copies) in the template. For low concentrations of target DNA, increase the number of cycles up to 40 cycles.

[3] Exact annealing temperature depends on the melting temperature of the primers.

[4] Elongation time depends on the length of target to be amplified. Use 1 minute per 1 kb of the PCR fragment. PCR product yield can be increased by using a cycle elongation feature. Usually 15 cycles are performed with a fixed elongation time, then 5 seconds are added to each of the remaining cycles. For example, cycle 15 is 45 seconds; cycle 16 is 50 seconds; cycle 17 is 55 seconds, etc.

Two-step RT-PCR

FastStart PCR Master can also be used to perform two-step RT-PCR.

- In two-step RT-PCR, the reverse transcription of RNA into cDNA is separated from the other reaction steps.
- Subsequent amplification of the first-strand cDNA is performed according to the standard PCR procedure, using the cDNA as the starting sample material. Use the Transcriptor First Strand cDNA Synthesis Kit* for reverse transcription of RNA into cDNA. Synthesize the cDNA using the Instructions for Use provided with the kit.

2.3. Parameters

Incorporation of Modified Nucleotides

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

PCR Cloning

TA cloning
FastStart PCR Master generates PCR products having 3′-single A overhangs. PCR fragment cloning into blunt-end vectors requires a polishing step in advance.

Working Concentration

2 U FastStart Taq DNA Polymerase per 50 µl reaction.

3. Results

Amplification of different DNA targets

The ability of the FastStart PCR Master to amplify different DNA targets (genomic DNA, plasmid DNA) without individual adjustment of the reagent compositions is demonstrated in Figure 1. The result shows that fragments ranging from 130 bp up to 1.1 kb can be obtained from complex genomic DNA in high yield and specificity. Amplification of even longer fragments (3.6 kb) is possible from plasmid DNA.

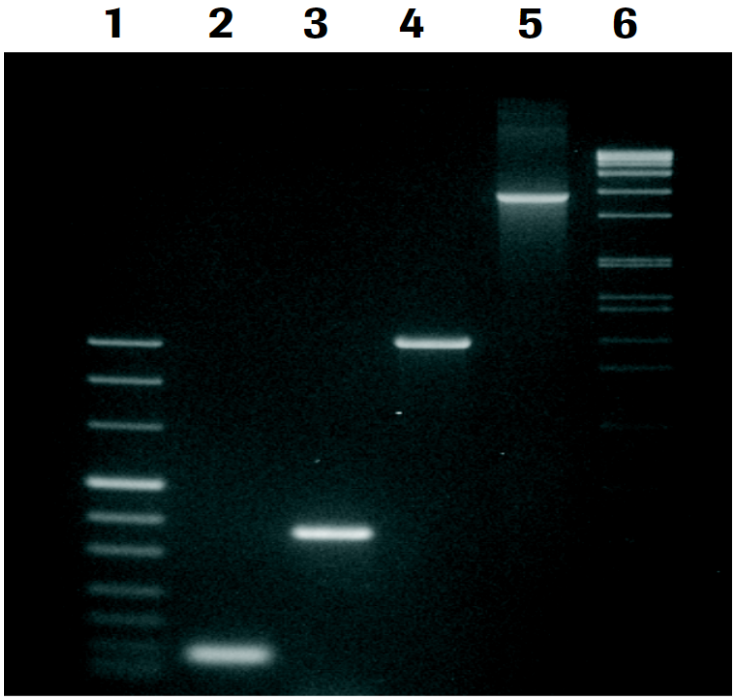

Fig 1: DNA fragments of different lengths were amplified under standard conditions.
Lane 1: Molecular Weight Marker VIII
Lane 2: FastStart PCR Master, 130 bp from 200 ng human genomic DNA.
Lane 3: FastStart PCR Master, 365 bp from 1 ng human genomic DNA.
Lane 4: FastStart PCR Master, 1.1 kb from 200 ng human genomic DNA.
Lane 5: FastStart PCR Master, 3.6 kb from 100 ng pUCIQ 17 plasmid.
Lane 6: Molecular Weight Marker VII

Amplification of cDNA

The ability of FastStart PCR Master to amplify cDNA is demonstrated in Figure 2.

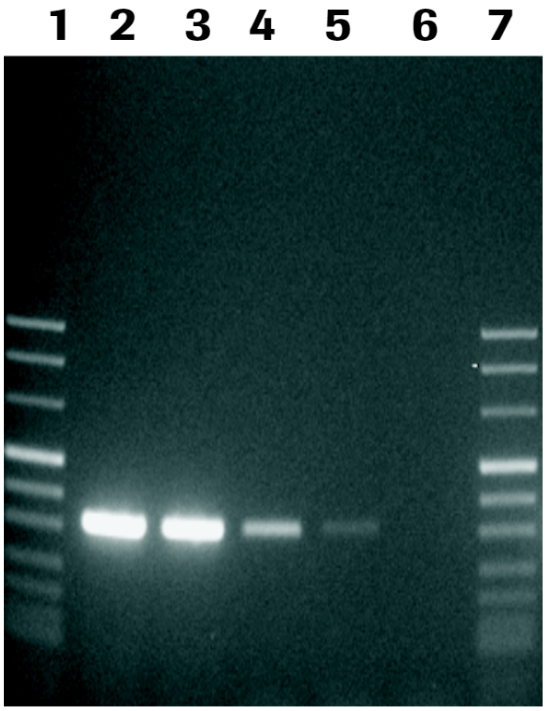

Fig. 2: A 324 bp fragment is amplified from cDNA template generated from human RNA using Transcriptor Reverse Transcriptase.
Lanes 1 and 7: Molecular Weight Marker VIII
Lane 2: FastStart PCR Master, 324 bp fragment, obtained from 100 ng RNA.
Lane 3: FastStart PCR Master, 324 bp fragment, obtained from 10 ng RNA.
Lane 4: FastStart PCR Master, 324 bp fragment, obtained from 1 ng RNA.
Lane 5: FastStart PCR Master, 324 bp fragment, obtained from 100 pg RNA.
Lane 6: FastStart PCR Master, 324 bp fragment, obtained from 0 pg RNA.

4. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	FastStart Taq DNA Polymerase not sufficiently activated.	Check if PCR was performed with an initial activation step at +95°C for 4 minutes. If high polymerase activity is needed in early cycles, you can sometimes improve results by extending the pre-incubation to 10 minutes.
		Check denaturation time during cycling; use 30 seconds at minimum.
		Check cycle number; increase the number of cycles in steps of 5 cycles.
	Pipetting errors	Repeat PCR.
		Check all concentrations and storage conditions of reagents.
	Unbalanced reaction	Check final concentrations of your components.
	DNA template problems.	Check quality and concentration of template: ▪ Analyze an aliquot on an agarose gel to check for possible degradation. ▪ Use serial dilution of template DNA. ▪ Test the template with an established primer pair or PCR system. ▪ Check or repeat template purification. ▪ Store template DNA at +2 to +8°C. For long-term storage, store at −15 to −25°C. ⚠ ***Avoid repeated freeze/thaw cycles.*** ▪ Use primers that amplify smaller genomic sequences.
	Cycle conditions not optimal.	Decrease annealing temperature.
		Increase cycle number.
		Check elongation time of 1 minute/1 kb PCR fragment.
		Denaturation time should not be lower than 30 seconds at +95°C.
	Primer design not optimal.	Design alternative primers.
	Primer concentration not optimal.	Titrate primer concentration (0.2 to 0.5 µM).
	Primer quality or storage problems.	If you use an established primer pair, check performance in an established PCR system, for example, with a control template.
		Make sure that the primers are not degraded.
		Always store primers at −15 to −25°C.
	Too high annealing temperature.	Reduce annealing temperature.
	Multiple contributing factors.	Test reaction with positive control template and primers of known performance.
		Use freshly made solutions of master mix, template, and primers.
Multiple bands or background smear.	Annealing temperature too low.	Increase annealing temperature.
	Primer design or concentration not optimal.	Review primer design.
		Titrate primer concentration (0.2 to 0.5 µM).
	Too many cycles.	Reduce cycles in steps of 3 cycles.
Problems specific to RT-PCR.	No product, additional bands, background smear observed.	The volume of cDNA template (RT reaction) should not exceed 10% of the final volume of the PCR reaction.
		Titrate cDNA template.
		Follow all troubleshooting tips.

5. Additional Information on this Product

5.1. Test Principle

FastStart Taq DNA Polymerase

FastStart PCR Master contains the FastStart Taq DNA Polymerase for hot start PCR to improve specificity and sensitivity of the PCR by minimizing the formation of nonspecific amplification products.

- This enzyme delivers excellent results thanks to its special enzyme design and optimized buffer system.
- FastStart Taq DNA Polymerase is a chemically modified form of thermostable recombinant Taq DNA polymerase that shows no activity up to +75°C. The enzyme is active only at high temperatures where primers no longer bind nonspecifically.
- The enzyme is completely activated by removal of blocking groups in a single pre-incubation step at +95°C for 4 minutes before cycling begins. Activation does not require the extra handling steps typical of other hot start techniques.

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

Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U 200 reactions of 20 µl final volume	03 531 287 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001

6.4. Trademarks

FASTSTART and MAGNA PURE 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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