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

FastStart High Fidelity PCR System

Version: 11
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Cat. No. 03 553 400 001	500 U 2 x 250 U, 5 U/µl 200 reactions in a final volume of 50 µl
Cat. No. 03 553 361 001	2,500 U 10 x 250 U, 5 U/µl 1,000 reactions in a final volume of 50 µl

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	FastStart High Fidelity PCR System, FastStart High Fidelity Enzyme Blend	Enzyme storage buffer: 20 mM Tris-HCl, pH 9.0 (+25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.2% Tween 20 (v/v), 50% glycerol (v/v).	03 553 400 001	2 vials, 50 µl each
			03 553 361 001	10 vials, 50 µl each
2	FastStart High Fidelity PCR System, FastStart High Fidelity Reaction Buffer, 10x conc. with 18 mM $MgCl_2$	For standard PCR.	03 553 400 001	2 vials, 1 ml each
			03 553 361 001	6 vials, 1 ml each
3	FastStart High Fidelity PCR System, FastStart High Fidelity Reaction Buffer, 10x conc. without $MgCl_2$	For dUTP incorporation and carryover prevention.	03 553 400 001	2 vials, 1 ml each
			03 553 361 001	6 vials, 1 ml each
4	FastStart High Fidelity PCR System, $MgCl_2$ 25 mM Stock Solution	For optimization of Mg^{2+} concentration.	03 553 400 001	2 vials, 1 ml each
			03 553 361 001	8 vials, 1 ml each
5	FastStart High Fidelity PCR System, DMSO	For difficult templates, such as GC-rich templates.	03 553 400 001	2 vials, 1 ml each
			03 553 361 001	6 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	FastStart High Fidelity Enzyme Blend	Store at −15 to −25°C.
2	FastStart High Fidelity Reaction Buffer, 10x conc. with 18 mM $MgCl_2$	
3	FastStart High Fidelity Reaction Buffer, 10x conc. without $MgCl_2$	
4	$MgCl_2$ 25 mM Stock Solution	
5	DMSO	

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*
- PCR Nucleotide Mix*

For DNA labeling with modified dNTPs

- Digoxigenin-11-dUTP, alkali-stabile*, or
- Digoxigenin-11-dUTP, alkali-labile*
- Biotin-16-dUTP*
- Fluorescein-12-dUTP*
- DIG DNA Labeling Mix*

For PCR for carryover prevention

- Uracil-DNA Glycosylase, heat-labile*
- PCR Nucleotide Mix^{PLUS*}

1.4. Application

The FastStart High Fidelity PCR System is an enzyme blend that combines all the benefits of FastStart Taq DNA Polymerase with 4-fold higher accuracy. It is useful for:

- Amplification of fragments up to 5 kb.
- Amplification experiments where a combination of high specificity, sensitivity, accuracy, and yield is needed.
- The detection of very rare messages and the amplification of fragments from limited amounts of sample material.
- Hot start PCR
- The incorporation of dUTP and subsequent decontamination via treatment with Uracil-DNA Glycosylase to prevent carryover contamination.
- Use in conjunction with robotics systems that perform the pipetting of the PCR setup at +15 to +25°C.
- Multiplex PCR
- Efficient labeling of DNA fragments with radioactive- or nonradioactive-labeled nucleotides.
- RT-PCR
- GC-RICH template amplification.
- Difficult, challenging PCRs.

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 5 to 250 ng complex genomic DNA or 100 pg to 10 ng plasmid DNA.

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

Primers

0.2 to 0.6 µM each primer (final concentration)
0.4 µM (final concentration) of each primer (standard concentration)

i *Prepare a 10x-concentrated solution, see section,* **Standard PCR**.

Mg^{2+} Concentration

1.4 to 4 mM (as $MgCl_2$) (optimal)
1.8 mM (as $MgCl_2$) (standard)

i *The Mg^{2+} concentration must be optimized when dUTP is used.*

General Considerations

The optimal conditions (incubation times and temperatures, concentrations of enzyme, template DNA, Mg^{2+}) depend on the system used and have to be determined individually.
As a starting point, use the following guidelines as mentioned below.

- Optimal enzyme concentration: 2.5 U/50 µl will usually produce satisfactory results.
- Optimal Mg^{2+} concentration can vary between 1.4 mM and 4 mM. In most cases, a Mg^{2+} concentration of 1.8 mM will produce satisfactory results if you use 200 µM of each dNTP.
- dNTP concentration: Always use equal concentrations of all four dNTPs. The most commonly used concentration is 200 µM.

Prevention of Carryover Contamination

dUTP incorporation and carryover prevention

The magnesium concentration must be optimized for each individual target.

- Use FastStart High Fidelity Reaction Buffer, 10x conc. without $MgCl_2$ (Vial 3) and $MgCl_2$, 25 mM Stock Solution (Vial 4) in concentrations ranging from 1.4 mM up to 4 mM final.
- Add the Uracil-DNA Glycosylase, heat-labile*, 2 U per reaction (2 µl).
- Use PCR Nucleotide Mix^{PLUS*}: 200 µM dATP, dCTP, dGTP, and 600 µM dUTP final concentration.

2.2. Protocols

Standard PCR

Preparation of PCR master mix

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

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

   If you are using, for example, the final concentration of 0.4 µM for each primer, the 10x-concentrated solution would contain a 4 µM concentration of the respective primer.

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

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	up to 50	–
FastStart High Fidelity Reaction Buffer, 10x conc. with $MgCl_2$ (Vial 2)[1,2]	5[1,2]	1.8 mM $MgCl_2$[1,2]
DMSO (Vial 5)[3] ⚠ ***Mix well after addition.***	0 – 5[3]	0 – 10%[3]
PCR Grade Nucleotide Mix*[1,2]	1[1,2]	200 µM of each dNTP[1,2]
Forward primer 1	5	0.4 µM
Reverse primer 2	5	0.4 µM
Template DNA	variable	5 – 250 ng (genomic DNA) 100 pg – 10 ng (plasmid DNA)
FastStart High Fidelity Enzyme Blend (Vial 1)	0.5	2.5 U/reaction
Total Volume	**50**	

4. Mix thoroughly and dispense appropriate volumes into thin-walled PCR tubes.

[1] See section, **Prevention of Carryover Contamination**.
[2] See section, **Incorporation of Modified Nucleotides**.
[3] For the optimization of a new assay, titrate the amount of DMSO in steps of 1 µl to determine the most optimal concentration.

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 either of the thermal profiles below to perform PCR.

   For carryover prevention, use the PCR Nucleotide Mix^{PLUS*} and 1 to 2 U of Uracil-DNA Glycosylase, heat-labile per reaction. Pre-incubate the reaction for 10 minutes at +20°C, then proceed with the PCR protocol.*

   Thermal Profile A for fragments up to 3 kb. Constant extension time.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	95	2 min	1
Denaturation Annealing Elongation	95 55 – 72 72	30 sec 30 sec 30 sec – 3 min[1]	35
Final Elongation	72	4 – 7 min	1
Cooling	4	indefinitely	

Thermal Profile B for fragments >3 kb. Gradually increasing extension time, ensuring a higher yield of amplification products.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation Annealing Elongation	94 55 – 68 68	10 – 30 sec 30 sec 3 – 5 min(1)	10
Denaturation Annealing Elongation	94 55 – 68(3) 68	10 – 30 sec 30 sec 3 – 5 min + 20 sec cycle elongation for each successive cycle(1)	30 (15 – 30)(2)
Final Elongation	68	7 min	1
Cooling	4	indefinitely	

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

(1) Elongation time depends upon length of the product to be amplified, approximately 1 minute per kb.
(2) Depending on template amount.
(3) Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.

2.3. Parameters

Error Rate

Approximately 4-fold higher fidelity compared to Taq DNA Polymerase and FastStart Taq DNA Polymerase. Fidelity determined with the *LacI* assay.

Incorporation of Modified Nucleotides

If you want to incorporate modified nonradioactive nucleotides, use the following concentrations and products:

DIG-dUTP*

- 10x DIG DNA Labeling Mix* 1:20: 0.1 mM DIG-dUTP, 1.9 mM dTTP, 2 mM dCTP, 2 mM dATP, and 2 mM dGTP.
- 10x DIG DNA Labeling Mix* 1:3: 0.35 mM DIG-dUTP, 0.65 mM dTTP, 1 mM dCTP, 1 mM dATP, and 1 mM dGTP.

Biotin-16-dUTP*

- 10x nucleotide mix: 0.7 mM Biotin-16-dUTP, 1.3 mM dTTP, 2 mM dCTP, 2 mM dATP, and 2 mM dGTP.

Fluorescein-12-dUTP*

- 10x PCR fluorescein labeling mix: 0.5 mM Fluorescein-12-dUTP, 1.5 mM dTTP, 2 mM dCTP, 2 mM dATP, and 2 mM dGTP.

⚠ ***A final $MgCl_2$ concentration of 1.8 mM can be used, however, optimize the concentration for each individual template/primer system.***

Maximum Fragment Size

Up to 5 kb.

PCR Cloning

TA cloning

Proofreading Activity

Yes

Temperature Optimum

+72°C (elongation) when amplifying fragments up to 3 kb.
For fragments >3 kb, use +68°C.

Volume Activity

5 U/µl

Working Concentration

2.5 U per 50 µl reaction (standard)

3. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	Pipetting errors	Check all concentrations and storage conditions of reagents.
	Difficult templates, such as GC-rich templates.	Add DMSO; titrate up to 10%. *Adding DMSO may negatively influence the accuracy of the reaction.*
		Use the GC-RICH PCR System*.
Problems with primers.	Primer design not optimal.	Design alternative primers.
	Primer concentration not optimal.	Both primers must have the same concentration.
		Titrate primer concentration (0.1 to 0.6 µM).
	Annealing temperature too high.	Reduce annealing temperature; minimal temperature is +55°C.
		Determine the optimal annealing temperature by touchdown PCR.
	Primer specificity not optimal.	Perform nested PCR with nested primers.
	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 the primers are not degraded.
		Always store primers in aliquots at −15 to −25°C.
	Formation of primer-dimers.	Use two reaction mixes for reaction setup: ▪ Mix 1: dNTPs, template DNA, and water up to 25 µl. ▪ Mix 2: all other components and water up to 25 µl. ▪ Combine Mixes 1 and 2, mix, centrifuge briefly, and immediately transfer the reactions into a thermal block cycler.
	DNA template problems.	Check quality and concentration of template: ▪ Analyze an aliquot on an agarose gel to check for possible degradation. ▪ Test the template with an established primer pair or PCR system. ▪ Check or repeat template purification.
	Enzyme concentration too low.	Increase the amount of FastStart Taq DNA Polymerase in 0.5 U steps up to 4 U per 50 µl reaction.
	$MgCl_2$ concentration too low.	Increase the $MgCl_2$ concentration in 0.25 mM steps; 1.8 mM $MgCl_2$ is the standard concentration.
	Cycle conditions not optimal.	Decrease annealing temperature.
		Increase cycle number.
		Make sure that the final elongation step was carried out.

Multiple bands or background smear.	Annealing temperature too low.	Increase annealing temperature according to the primer length.
	Primer design or concentration not optimal.	Review primer design.
		Titrate primer concentration (0.1 to 0.6 µM).
		Both primers must have the same concentration and identical annealing temperatures.
		Perform nested PCR with nested primers.
	Difficult templates, such as GC-rich templates.	Add DMSO; titrate up to 10%.
		Use the GC-RICH PCR System*.
	DNA template problems.	Use serial titration/dilution of template to avoid the influence of potential PCR inhibitors.
PCR products in negative control experiments.	Carryover contamination	Exchange all reagents, especially water.
		Use aerosol-resistant pipette tips.
		Set up PCR run in an area separate from that used for PCR product analysis.
		Use dUTP* (600 µM) instead of dTTP (200 µM) and Uracil-DNA Glycosylase, heat-labile* (2 U/50 µl reaction); also, increase Mg^{2+} concentration up to 4 mM at most.
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.

4. Additional Information on this Product

4.1. Test Principle

Background information

The FastStart High Fidelity PCR System is an enzyme blend of chemically modified Taq DNA Polymerase–FastStart Taq DNA Polymerase–and a thermostable (via chemical modification) proofreading protein. This protein mediates proofreading activity, but has no polymerase activity itself. This enzyme blend is inactive at temperatures below +75°C and does not elongate non-specific primer-template hybrids that may form during PCR set-up. After activation via a 2 min heat incubation step at +95°C, the processivity of FastStart Taq DNA Polymerase and the ability of the proofreading protein to excise incorrectly incorporated nucleotides combine to provide hot start amplification of all kinds of DNA and cDNA targets up to 5 kb with

- High specificity, sensitivity and yield
- Approximately 4-fold increased fidelity compared to Taq DNA.

4.2. Quality Control

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

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
PCR Nucleotide Mix^{PLUS}	2 x 100 µl, 200 PCR reactions in 50 µl	11 888 412 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
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
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
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
GC-RICH PCR System	100 U, 50 reactions in a final volume of 50 µl	12 140 306 001
DIG DNA Labeling Mix	50 µl, 10x conc. 25 standard reactions	11 277 065 910

5.4. Trademarks

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

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany