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

FastStart Taq DNA Polymerase, 5 U/µl, dNTPack

Version: 12
Content Version: November 2025

FastStart Taq DNA Polymerase with additional ready-to-use 10 mM PCR Grade Nucleotide Mix.

Cat. No. 04 738 357 001	500 U 2 x 250 U 250 reactions in a final volume of 50 µl
Cat. No. 04 738 381 001	1,000 U 4 x 250 U 500 reactions in a final volume of 50 µl
Cat. No. 04 738 403 001	2,500 U 10 x 250 U 1,250 reactions in a final volume of 50 µl
Cat. No. 04 738 420 001	5,000 U 20 x 250 U 2,500 reactions in a final volume of 50 µl

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Function / description	Catalog number	Content
1	colorless	FastStart Taq DNA Polymerase, dNTPack FastStart Taq DNA Polymerase	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).	04 738 357 001	2 vials, 50 µl each
				04 738 381 001	4 vials, 50 µl each
				04 738 403 001	10 vials, 50 µl each
				04 738 420 001	20 vials, 50 µl each
2	green	FastStart Taq DNA Polymerase, dNTPack PCR buffer, 10x conc. with $MgCl_2$	▪ Buffer composition: 500 mM Tris-HCl, 100 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$, pH 8.3 (+25°C). ▪ PCR buffer with 20 mM $MgCl_2$.	04 738 357 001	2 vials, 1 ml each
				04 738 381 001	3 vials, 1 ml each
				04 738 403 001	7 vials, 1 ml each
				04 738 420 001	14 vials, 1 ml each
3	yellow	FastStart Taq DNA Polymerase, dNTPack PCR buffer, 10x conc. without $MgCl_2$	Buffer composition: 500 mM Tris-HCl, 100 mM KCl, 50 mM $(NH_4)_2SO_4$, pH 8.3 (+25°C).	04 738 357 001	2 vials, 1 ml each
				04 738 381 001	3 vials, 1 ml each
				04 738 403 001	7 vials, 1 ml each
				04 738 420 001	14 vials, 1 ml each
4	blue	FastStart Taq DNA Polymerase, dNTPack $MgCl_2$ 25 mM Stock Solution	For optimization of Mg^{2+} concentration.	04 738 357 001	2 vials, 1 ml each
				04 738 381 001	4 vials, 1 ml each
				04 738 403 001	10 vials, 1 ml each
				04 738 420 001	20 vials, 1 ml each
5	red	FastStart Taq DNA Polymerase, dNTPack GC-RICH solution, 5x conc.	For amplification of difficult templates.	04 738 357 001	3 vials, 1 ml each
				04 738 381 001	5 vials, 1 ml each
				04 738 403 001	13 vials, 1 ml each
				04 738 420 001	26 vials, 1 ml each

6	purple	FastStart Taq DNA Polymerase, dNTPack PCR Grade Nucleotide Mix	Ready-to-use solution, 10 mM each dNTP	04 738 357 001	2 vials, 200 µl each
				04 738 381 001	4 vials, 200 µl each
				04 738 403 001	10 vials, 200 µl each
				04 738 420 001	20 vials, 200 µl 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	Cap	Label	Storage
1	colorless	FastStart Taq DNA Polymerase	Store at −15 to −25°C.
2	green	PCR buffer, 10x conc. with $MgCl_2$	
3	yellow	PCR buffer, 10x conc. without $MgCl_2$	
4	blue	$MgCl_2$ 25 mM Stock Solution	
5	red	GC-RICH solution, 5x conc.	
6	purple	PCR Grade Nucleotide Mix	

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 and PCR using GC-RICH solution

- PCR primers
- Template DNA
- Water, PCR Grade*

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

FastStart Taq DNA Polymerase is an ideal tool for hot start PCR since the enzyme remains inactive during PCR setup and prior to the initial denaturation step. Since it is inactive at low temperatures, FastStart Taq DNA Polymerase cannot elongate nonspecific primer-template hybrids that may form at those temperatures.

- Amplification of genomic DNA and cDNA targets up to 3 kb with high specificity, sensitivity, and yield.
- Multiplex PCR
- Difficult templates, such as secondary structures or GC-rich sequences.
- Automated PCR, such as setup and handling at +15 to +25°C.

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 10 pg to 500 ng complex genomic DNA or 10 pg to 100 ng plasmid DNA/cDNA.

Primers

0.2 to 0.5 µM each (final concentration)
0.2 µM each (standard concentration)

Mg^{2+} Concentration

1 to 4 mM (as $MgCl_2$) (optimal)
2 mM (as $MgCl_2$) (standard)

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg^{2+} vary from system to system and must be determined for each individual experimental system.
As a starting point, use the following guidelines:

- Optimal enzyme concentration: 0.5 to 5 U/50 µl. A concentration of 2 U/50 µl will usually produce satisfactory results.
- Optimal Mg^{2+} concentration can vary between 1 mM and 4 mM. In most cases, a Mg^{2+} concentration of 2 mM will produce satisfactory results if you use 200 µM of each dNTP.
- dNTP concentration: Always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 100 and 500 µM; the most commonly used concentration is 200 µM. If you increase the dNTP concentration, you must also increase the Mg^{2+} concentration.

Two different procedures are described:

- Standard PCR procedure.
- PCR procedure using GC-RICH solution.

The protocols are designed for a final 50 µl reaction volume. For other volumes, the reaction and cycle conditions have to be optimized.

FastStart Taq DNA Polymerase versus Taq DNA Polymerase

The major differences of a typical PCR when using FastStart Taq DNA Polymerase include:
- Increased denaturation time prior to PCR of approximately 4 minutes (2 to 6 minutes) at +95°C.
- Minimal denaturation time of 30 seconds in each cycle is required.
- Standard Mg^{2+} concentration is 2 mM.

All other conditions, such as dNTPs, primers, template concentrations, and cycle number are identical.

GC-RICH solution

The optimal concentration of GC-RICH solution is 10 µl per 50 µl assay. When using the GC-RICH solution the first time for a particular primer-template pair, always perform parallel reactions with and without GC-RICH solution.

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 0.2 µM for each primer, the 10x-concentrated solution would contain a 2 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	variable	–
PCR buffer, 10x conc. with $MgCl_2$ [1] (Vial 2)	5	2 mM $MgCl_2$
$MgCl_2$ 25 mM Stock Solution[2] (Vial 4)	variable	1.5 – 4 mM
PCR Grade Nucleotide Mix (Vial 6)	1	200 µM of each dNTP
Forward primer 1	5	0.2 – 1 µM
Reverse primer 2	5	0.2 – 1 µM
FastStart Taq DNA Polymerase (Vial 1)	0.4	2 U
Template DNA *Added in Step 5.*	variable	100 – 250 ng genomic DNA/reaction
Total Volume	**50**	

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

5. Add template DNA to the individual tubes containing the master mix.

6. Mix each PCR tube well to produce a homogeneous solution.
   – Shake down or centrifuge briefly to collect the solution at the bottom of the tube.

[1] Contains 20 mM $MgCl_2$; if Mg concentration needs to be titrated, use 10x PCR buffer without $MgCl_2$ (Vial 3).
[2] Use if Mg^{2+}-titration is required.

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/activation	95	4 min(1)	1
Denaturation Annealing Elongation	95 45 – 65(3) 72	30 sec 30 sec 45 sec – 3 min(4)	30 – 40(2)
Final Elongation	72	7 min	1
Cooling	4	indefinitely	

2. Analyze the samples on a 1 to 2% agarose gel.

(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 (14-band multiplexing PCR with 28 primers was tested) might be increased by longer activation time up to 10 minutes or more cycles. Activation times down to 2 minutes will give good results.

(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.

PCR using GC-RICH solution

Preparation of PCR master mix

⚠ ***When using the GC-RICH solution (Vial 5) the first time for a particular primer-template pair, always perform parallel reactions with and without GC-RICH solution.***

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.

   When using, for example, a final concentration of 0.2 µM of each primer, prepare a 10x-concentrated solution containing 2 µM 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	variable	–
PCR buffer, 10x conc. with $MgCl_2$(1) (Vial 2)	5	2 mM $MgCl_2$
$MgCl_2$ 25 mM Stock Solution(2) (Vial 4)	variable	1.5 – 4 mM
GC-RICH solution, 5x conc. (Vial 5)	10	1x
PCR Grade Nucleotide Mix (Vial 6)	1	200 µM (of each dNTP)
Forward primer 1	5	0.2 – 1 µM
Reverse primer 2	5	0.2 – 1 µM
FastStart Taq DNA Polymerase (Vial 1)	0.4	2 U
Template DNA *Added in Step 5.*	variable	up to 500 ng/reaction
Total Volume	**50**	

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

5 Add template DNA to the individual tubes containing the master mix.

6 Mix each PCR tube well to produce a homogeneous solution.
– Shake down or centrifuge briefly to collect the solution at the bottom of the tube.

(1) Contains 20 mM $MgCl_2$; if Mg^{2+} concentration needs to be titrated, use 10x PCR buffer without $MgCl_2$ (Vial 3).
(2) Use if Mg^{2+}-titration is required.

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/activation	95	4 min(1)	1
Denaturation Annealing Elongation	95 45 – 65(3) 72	30 sec 30 sec 45 sec – 3 min(4)	30 – 40(2)
Final Elongation	72	7 min	1
Cooling	4	indefinitely	

2 Analyze the samples on a 1 to 2% agarose gel.

(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 (14-band multiplexing PCR with 28 primers was tested) might be increased by longer activation time up to 10 minutes or more cycles. Activation times down to 2 minutes will give good results.
(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.

2.3. Parameters

Incorporation of Modified Nucleotides

For labeling of PCR products, modified dNTPs, such as DIG-11- dUTP*, Biotin-16-dUTP*, Fluorescein-12-dUTP* are typically used in a ratio together with dTTP.

- For Southern blot applications, the respective concentration is 134 µM dTTP and 66 µM DIG-11-dUTP.
- For ELISA, the respective concentration is 190 µM dTTP and 10 µM DIG-11-dUTP.

PCR Cloning

TA cloning
For cloning into blunt-end vectors, an additional end-polishing step is required.

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

0.5 to 5 U per 50 µl reaction (optimal)
2 U per 50 µl reaction (standard)

3. Results

Standard PCR

Sensitivity

To demonstrate the sensitivity of FastStart Taq DNA Polymerase, a 365 bp fragment of the human t-PA gene was amplified using various concentrations of human genomic DNA (Figure 1). PCR was performed in a 50 µl reaction using 2 U of FastStart Taq DNA Polymerase under standard conditions (200 µM each dNTP, 200 nM each primer, 2 mM $MgCl_2$) with 3 ng (lane 1), 1 ng (lane 2), 500 pg (lane 3), 300 pg (lane 4),150 pg (lane 5), 60 pg (lane 6), 30 pg (lane 7), 10 pg (lane 8) human genomic DNA, and no template control (lane 9). After 40 cycles with an initial 2 minutes denaturation/ activation step, a specific PCR product was detectable with 10 pg of starting human genomic DNA.

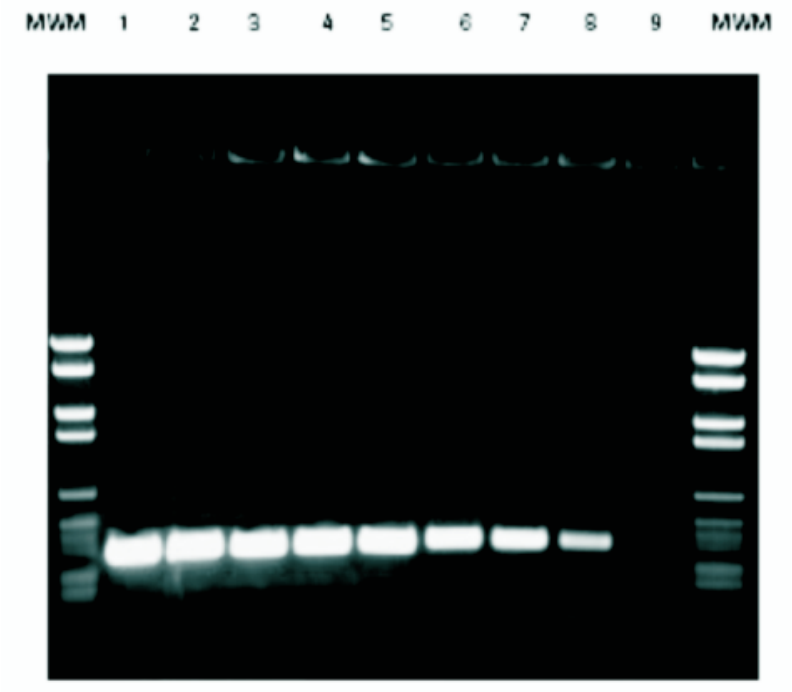

Fig. 1: Amplification of 365 bp tPA fragment down to 10 pg human genomic DNA which is equivalent to 3 gene copies (3 pg is equivalent to 1 copy).

Specificity

Specificity of FastStart Taq DNA Polymerase was compared to Taq DNA polymerase by amplifying a 130 bp fragment of the human t-PA gene (Figure 2). For both enzymes, standard PCR conditions were applied (2 U/ 50 µl reaction with respective buffer conditions). 100 ng (lanes 1 and 7), 50 ng (lanes 2 and 8), 10 ng (lanes 3 and 9), 5 ng (lanes 4 and 10), and without human genomic DNA (lanes 5 and 11) was amplified (30 cycles with identical cycle program for both enzymes) and product visualized on an agarose gel. With FastStart Taq DNA Polymerase, a single specific PCR product was obtained (lanes 7 to 10); with Taq DNA polymerase, nonspecific PCR products as well as lower sensitivity was obtained (lanes 1 to 5).

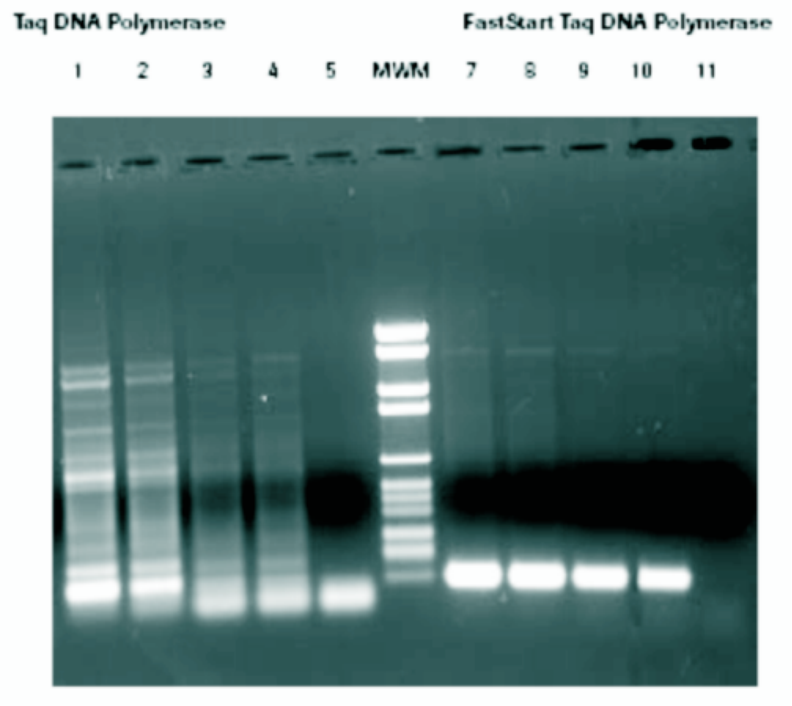

Fig. 2: Highly specific PCR using the hot start capability of FastStart Taq DNA Polymerase.

PCR using the GC-RICH solution

Sensitivity

GC-RICH solution changes the melting behavior of DNA and can be used for primer template pairs with high GC-content that do not work well with standard conditions. To compare the ability of the GC-RICH solution, FastStart Taq DNA Polymerase was used to amplify a 284 bp human ApoE gene product with and without the additive (Figure 3). Out of 200 ng human genomic DNA and 35 cycles, a specific PCR product is visible when the GC-RICH solution is used (lane 3). Without this additive, PCR fails as demonstrated on FastStart Taq DNA Polymerase alone (lane 2), Taq DNA Polymerase (lane 7), or competitor's A and B hot start Taq DNA polymerase (lanes 4 and 6). Competitor A Taq DNA Polymerase with specific buffer (lane 5) also facilitates amplification of this target.

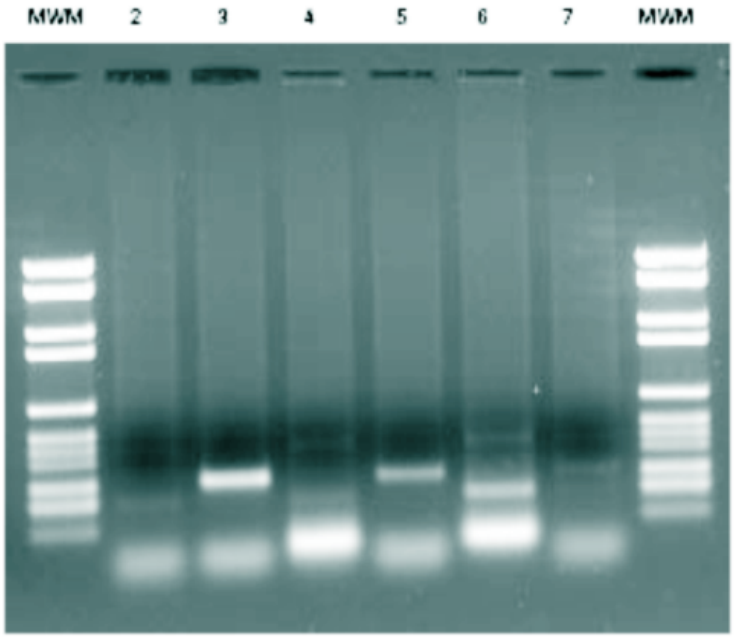

Fig. 3: Amplification of a 284 bp human ApoE gene fragment (GC content 74%).
Lane 2: FastStart Taq DNA Polymerase
Lane 3: FastStart Taq DNA Polymerase + GC-RICH solution
Lane 4: Competitor A
Lane 5: Competitor A plus special buffer
Lane 6: Competitor B
Lane 7: Taq DNA Polymerase

4. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	FastStart Taq DNA Polymerase not sufficiently activated.	Check if PCR was started with previous activation step at +95°C for 4 minutes. Alternatively, use 10 minutes.
		Check denaturation time during cycles; it should be at least 30 seconds.
		Check cycle numbers; increase the number of cycles in steps of 5 cycles.
	Pipetting errors	Repeat PCR.
		Check all concentrations and storage conditions of reagents.
	Difficult templates, such as GC-rich templates.	Repeat PCR under same conditions and add GC-RICH solution, see section, **Protocols, PCR using GC-RICH Solution**.
		If performance is still not acceptable, titrate GC-RICH solution (4, 6, 8 µl), reduce or increase annealing temperature, titrate Mg^{2+} concentration and/or enzyme concentration.
	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. ▪ Use serial dilution of template.
	Enzyme concentration too low.	Use 2 U FastStart Taq DNA Polymerase per 50 µl reaction.
		If necessary, increase the amount of polymerase in 0.5 U steps.
	$MgCl_2$ concentration not optimal.	Titrate the Mg^{2+} concentration from 1 to 4 mM in 0.5 mM steps with PCR Buffer, 10x conc. without $MgCl_2$ (Vial 3).
	Cycle conditions not optimal.	Decrease annealing temperature.
		Increase cycle number.
		Check elongation time (1 minute/1 kb PCR fragment).
		Denaturation time must not be <30 seconds at +95°C.
Little or no PCR product	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.
	Primers problems due to too high annealing temperature,	Reduce annealing temperature.

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).
	Difficult templates, such as GC-rich templates.	Perform PCR with the GC-RICH solution.
	Starting concentration of Mg^{2+} ions too high.	Reduce Mg^{2+} concentration.
	Starting concentration of template versus cycles too high.	Check template concentration by titration or by gel electrophoresis.
	Starting concentration of enzyme too high.	Use 2 U of FastStart Taq DNA Polymerase per 50 µl reaction.
		Titrate enzyme units down in steps of 0.25 U.
Problems with cloning of PCR products.	FastStart Taq DNA Polymerase adds additional A at the 3′ end of PCR products similar to Taq DNA Polymerase. Therefore, PCR products can be cloned into TA cloning vectors.	Cloning in blunt-end vectors need a blunt-end polishing step first.
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

Background information

FastStart Taq DNA Polymerase has been developed by Roche to increase specificity and sensitivity of PCR in a convenient and rapid way. With FastStart Taq DNA Polymerase, hot start PCR can be applied to genomic DNA and cDNA templates, eliminating extra handling steps or additional time required, typical of other known hot start techniques.

FastStart Taq DNA Polymerase is a thermostable, chemically modified form of recombinant Taq DNA Polymerase. The enzyme is active only at high temperatures where primers no longer bind non-specifically. The enzyme is completely activated by removal of blocking groups in a single pre-incubation step (95°C, 4 minutes) before cycling begins.

The combination of FastStart Taq DNA Polymerase and the optimized PCR buffer minimizes non-specific amplification products and primer dimers allowing highest sensitivity. The provided GC-RICH solution, a PCR additive that facilitates amplification of difficult templates by modifying the melting behavior, will improve PCR performance on templates rich in secondary structures or GC content.

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

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
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

6.4. Trademarks

MAGNA PURE and FASTSTART 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:
Product Disclaimers.

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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