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

Print Protocol | Print Changes to Previous Version | Print License Disclaimer

FastStart Taq DNA Polymerase, 5 U/µl

Version 13

Content version: April 2016

Cat. No. 12 032 902 001	100 U for 50 PCR reactions
Cat. No. 12 032 929 001	2 × 250 U for 250 PCR reactions
Cat. No. 12 032 937 001	4 × 250 U for 500 PCR reactions
Cat. No. 12 032 945 001	10 × 250 U for 1,250 PCR reactions
Cat. No. 12 032 953 001	20 × 250 U for 2,500 PCR reactions

Store the kit at −15 to −25°C

sigma-aldrich.com

Table of Contents

PROTOCOL

PROTOCOL

1. What this Product Does

Number of Reactions

For a typical test, 2 U of FastStart Taq DNA Polymerase are used in a 50 µl reaction volume. The number of tests depend on the pack size ordered.

Contents

Vial	Label	Contents A) Cat. No. 12 032 902 001 B) Cat. No. 12 032 929 001 C) Cat. No. 12 032 937 001 D) Cat. No. 12 032 945 001 E) Cat. No. 12 032 953 001
1 colorless cap	FastStart Taq DNA Polymerase (5 U/µl)	A) 1 × 20 µl; B) 2 × 50 µl; C) 4 × 50 µl; D) 10 × 50 µl; E) 20 × 50 µl • 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)]
2 green cap	PCR reaction buffer, 10× conc. with 20 mM $MgCl_2$	A) 1 × 1 ml; B) 2 × 1 ml; C) 3 × 1 ml; D) 7 × 1 ml; E) 14 × 1 ml • 500 mM Tris/HCl, 100 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$, pH 8.3/ +25°C
3 yellow cap	PCR reaction buffer, 10× conc. without $MgCl_2$	A) 1 × 1 ml; B) 2 × 1 ml; C) 3 × 1 ml; D) 7 × 1 ml; E) 14 × 1 ml • 500 mM Tris/HCl, 100 mM KCl, 50 mM $(NH_4)_2SO_4$, pH 8.3/ +25°C
4 blue cap	$MgCl_2$ stock solution, 25 mM	A) 1 × 1 ml; B) 2 × 1 ml; C) 4 × 1 ml; D) 10 × 1 ml; E) 20 × 1 ml
5 red cap	GC-RICH solution, 5× conc.	A) 1 × 1 ml; B) 3 × 1 ml; C) 5 × 1 ml; D) 13 × 1 ml ; E) 26 × 1 ml

Storage and Stability

The undiluted solutions are stable when stored at –15 to –25°C until the control date printed on the label.

Additional Equipment and Reagents Required

- Template DNA, gene-specific primer pair
- Water, PCR Grade*
- Thermal block cycler (*e.g.,* Applied Biosystems GeneAmp PCR System 9600)
- 0.2 ml thin-walled PCR tubes
- Sterile reaction tubes for preparing master mixes and dilutions
- Nucleotides, PCR Grade*, PCR Nucleotide Mix* or PCR Nucleotide Mix^{PLUS}* (contains dUTP for carry-over prevention)

Application

FastStart Taq DNA Polymerase is an ideal tool for hot start PCR, because the enzyme remains inactive during PCR set-up and prior to the initial denaturation step. Since it is inactive at low temperatures, FastStart Taq DNA Polymerase cannot elongate non-specific 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 *e.g.,* secondary structures or GC-rich sequences
- Automated PCR *e.g.,* set-up and handling at room temperatures
- Carry-over prevention (additionally required: PCR Nucleotide Mix^{PLUS}* and Uracil-DNA Glycosidase, heat-labile*)

Enzyme Properties

Volume Activity	5 U/µl
Optimal Enzyme Concentration	Varies between 0.5 and 5 U per 50 µl assay, the recommended starting concentration is 2 U per 50 µl assay.
Optimal Elongation Temperature	The elongation temperature is +72°C when amplifying fragments up to 3 kb. When amplifying fragments larger than 3 kb, +68°C might be favourable.
Optimal $MgCl_2$ Concentration	Varies between 1 - 4 mM, the recommended starting concentration is 2 mM.
Primers	Use primers at a final concentration of 0.2 - 0.5 µM each. A recommended starting concentration is 0.2 µM each.
PCR Cloning	T/A cloning. For cloning into blunt end vectors an additional end polishing step is needed. (Refer *e.g.,* to PCR Cloning Kit*).

2. How To Use this Product

2.1 Before You Begin

General Considerations

The optimal reaction conditions (incubation times and temperatures, concentration of FastStart Taq DNA Polymerase, template DNA, Mg^{2+}-ions) depend on the template and primer pair and must be determined individually.

Two different procedures are described.

- Procedure A: standard PCR procedure
- Procedure B: PCR procedure using GC-RICH solution
- Procedure C: PCR procedure for carry-over prevention

⚠ 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

The major differences of a typical PCR procedure using FastStart Taq DNA Polymerase to a PCR using standard Taq DNA polymerase are

- increased denaturation time prior to PCR of around 4 min (2 - 6 min) at +95°C
- a minimal denaturation time of 30 sec in each cycle is required
- standard Mg^{2+} concentration is 2 mM.

All other conditions - 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.

dNTP Concentration

The optimal concentration of dNTPs (dATP, dGTP, dCTP, dTTP) range from 0.1 – 0.5 mM. The recommended concentration is 0.2 mM.

For carry-over prevention 0.2 mM dTTP is substituted by 0.6 mM dUTP.

For labeling of PCR products modified dNTPs (*e.g.,* DIG-11- dUTP, Biotin-16-dUTP, Fluorescein-12-dUTP) are typically used in a ratio together with dTTP. For Southern blot application the respective concentration is 134 µM dTTP and 66 µM DIG-11-dUTP, for ELISA application the respective concentration is 190 µM dTTP and 10 µM DIG-11-dUTP.

Sample Material

Every sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors can be used. Typically 10 pg – 500 ng human genomic DNA or 10 pg – 100 ng cDNA or plasmid are used.

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

❷ To a sterile reaction tube on ice, add the components in the order listed below: (for each 50 µl reaction)

Component	Vol.	Final conc.
Water, PCR Grade	variable	
10 × PCR Buffer[1)] (vial 2)	5 µl	2 mM $MgCl_2$
$MgCl_2$ Solution[2)], 25 mM (vial 4)	variable	1.5 – 4 mM
10 mM dATP, PCR grade[3)]	1 µl	200 µM
10 mM dCTP, PCR grade[3)]	1 µl	200 µM
10 mM dGTP, PCR grade[3)]	1 µl	200 µM
10 mM dTTP, PCR grade[3)]	1 µl	200 µM
Upstream primer	5 µl	0.2 – 1 µM
Downstream primer	5 µl	0.2 – 1 µM
FastStart Taq DNA Polymerase (vial 1)	0.4 µl	2 U
Read step 3 and 4		
Template DNA, added at step 4	variable	up to 500 ng/ reaction
Total volume	**50 µl**	

[1)]contains 20 mM $MgCl_2$; if Mg concentration should be titrated use 10× PCR buffer without $MgCl_2$, vial 3 (yellow cap)
[2)]only if Mg-titration is required
[3)] alternatively 1 µl of 10 mM PCR Nucleotide Mix* can be used

❸ Mix thoroughly and dispense appropriate volumes into PCR tubes (preferably thin-walled PCR tubes)

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

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

❻ Place your sample in a thermal block cycler and perform PCR.
An example for a cycle profile is given for the Applied Biosystems GeneAmp PCR System 9600. When using other thermal block cyclers the cycle conditions have to be adjusted.

❼ **PCR reaction:** A typical temperature profile is given for the Applied Biosystems GeneAmp PCR System 9600

	Cycles	Time	Temp
Denaturation/Activation	1	4 min[a)]	95°C
Denaturation Annealing Elongation	30 - 40[d)]	30 s 30 s 45 s – 3 min[c)]	95°C 45 to 65°C[b)] 72°C
Final Extension	1	7 min	72°C
Cooling		unlimited time	4°C

a) 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 min or more cycles. Activation times down to 2 min 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 min or more cycles. Activation times down to 2 min will give good results.
b) Exact annealing temperature depends on the melting temperature of the primers.
c) Elongation time depends on the length of target to be amplified. Recommended time is 1 min 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 e.g., cycle 15 = 45 sec; cycle 16 = 50 sec; cycle 17 = 55 sec etc.
d) 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.

❽ Analyze the samples on a 1 - 2% agarose gel.

⚠ 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.

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

❷ To a sterile reaction tube on ice, add the components in the order listed below:
(For each 50 µl reaction)

Component	Vol.	Final conc.
Water, PCR Grade	variable	
10 × PCR Buffer[1)] (vial 2)	5 µl	2 mM $MgCl_2$
$MgCl_2$ Solution[2)], 25 mM (vial 4)	variable	1.5 – 4 mM
GC-RICH solution (5 ×) (vial 5)	10 µl	1 ×
10 mM dATP, PCR grade[3)]	1 µl	200 µM
10 mM dCTP, PCR grade[3)]	1 µl	200 µM
10 mM dGTP, PCR grade[3)]	1 µl	200 µM
10 mM dTTP, PCR grade[3)]	1 µl	200 µM
Upstream primer	5 µl	0.2 – 1 µM
Downstream primer	5 µl	0.2 – 1 µM
FastStart Taq DNA Polymerase (vial 1)	0.4 µl	2 U
Read step 3 and 4		
Template DNA, added at step 4	variable	up to 500 ng/reaction
Total volume	**50 µl**	

[1)]contains 20 mM $MgCl_2$; if Mg concentration should be titrated use 10× PCR buffer without $MgCl_2$, vial 3 (yellow cap)
[2)]only if Mg-titration is required
[3)] alternatively 1 µl of 10 mM PCR Nucleotide Mix can be used

❸ Mix thoroughly and dispense appropriate volumes into PCR tubes (preferably thin-walled PCR tubes)

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

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

❻ Place your sample in a thermal block cycler and perform PCR.
An example for a cycle profile is given for the Applied Biosystems GeneAmp PCR System 9600. When using other thermal block cyclers the cycle conditions have to be adjusted.

❼ **PCR reaction:** A typical temperature profile is given for the Applied Biosystems GeneAmp PCR System 9600

	Cycles	Time	Temp
Denaturation/Activation	1	4 min [a)]	95°C
Denaturation	30 – 40 [d)]	30 s	95°C
Annealing		30 s	45 to 65°C [b)]
Elongation		45 s – 3 min [c)]	72°C
Final Extension	1	7 min	72°C
Cooling		unlimited time	4°C

a) 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 min or more cycles. Activation times down to 2 min 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 min or more cycles. Activation times down to 2 min will give good results.
b) Exact annealing temperature depends on the melting temperature of the primers.
c) Elongation time depends on the length of target to be amplified. Recommended time is 1 min 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 e.g., cycle 15 = 45 sec; cycle 16 = 50 sec; cycle 17 = 55 sec etc.
d) 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.

❽ Analyze the samples on a 1 - 2% agarose gel.

⚠ Additionally required: PCR Nucleotide Mix^{PLUS*} and Uracil-DNA Glycosylase, heat-labile*.

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

❷ To a sterile reaction tube on ice, add the components in the order listed below:
(For each 50 µl reaction)

Component	Vol.	Final conc.
Water, PCR Grade	variable	
10 × PCR Buffer (vial 2)	5 µl	2 mM $MgCl_2$
$MgCl_2$ Solution[1)], 25 mM (vial 4)	variable	1.5 – 4 mM
PCR Nucleotide Mix^{Plus}	1 µl	200 µM (dATP, dCTP, dGTP), 600 µM dUTP
Upstream primer	variable	0.2 – 1 µM
Downstream primer	variable	0.2 – 1 µM
Heat-labile UNG (1 U/µl)	1 µl	1 U
FastStart Taq DNA Polymerase (vial 1)	0.4 µl	2 U
Read step 3 and 4		
Template DNA, added at step 4	variable	up to 500 ng/reaction
Total volume	**50 µl**	

[1)]The optimal Mg^{2+}-ions concentration depends on primer pairs and template. For best results determine optimal Mg-ions concentration empirically using 0.5 mM titration steps. When using 600 µM dUTP increase the $MgCl_2$ concentration to 2.5 mM.

❸ Mix thoroughly and dispense appropriate volumes into PCR tubes (preferably thin-walled PCR tubes)

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

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

❻ Place your sample in a thermal block cycler and perform PCR.
An example for a cycle profile is given for the Applied Biosystems GeneAmp PCR System 9600. When using other thermal block cyclers the cycle conditions have to be adjusted.

❼ **PCR reaction:** A typical temperature profile is given for the Applied Biosystems GeneAmp PCR System 9600

continued on next page

	Cycles	Time	Temp
UNG incubation	1×	10 min	20°C
Inactivation of UNG/ Denaturation of template/ Activation of polymerase	1×	4 min [a]	95°C
Denaturation Annealing Elongation	30 – 40 [d]	30 s 30 s 45 s – 3 min [c]	95°C 45 to 65°C [b] 72°C
Final Extension	1	7 min	72°C
Hold	1×	up to 8 h [e]	4°C

[a] This step inactivates the UNG, 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 min or more cycles. Activation times down to 2 min 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 min or more cycles. Activation times down to 2 min will give good results. Uracil-DNA Glycosylase, heat-labile, BMTU 3346 rec, is inactivated completely and permanently by heating to +95 °C for 2 minutes.

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

[c] Elongation time depends on the length of target to be amplified. Recommended time is 1 min 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 e.g., cycle 15 = 45 sec; cycle 16 = 50 sec; cycle 17 = 55 sec etc.

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

[e] Uracil-DNA Glycosylase, heat-labile, from BMTU 3346, recombinant, does not recover activity after inactivation. PCR product containing dUTP can be stored at +4 to +8 °C for several hours. For long-term storage freeze at -15 to -25°C.

8 Analyze the samples on a 1 - 2% agarose gel.

3. Results

3.1 Typical Results using the Standard PCR Procedure

Sensitivity

To demonstrate the sensitivity of FastStart Taq DNA Polymerase a 365 bp fragment of the human tPA gene (single copy gene) was amplified using various concentrations of human genomic DNA (Figure 1).

PCR has been performed in a 50 µl reaction using 2 U of FastStart Taq DNA Polymerase under standard conditions [200 µM dNTP (each), 200 nM primer (each), 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 is detectable down to 10 pg of human genomic DNA.

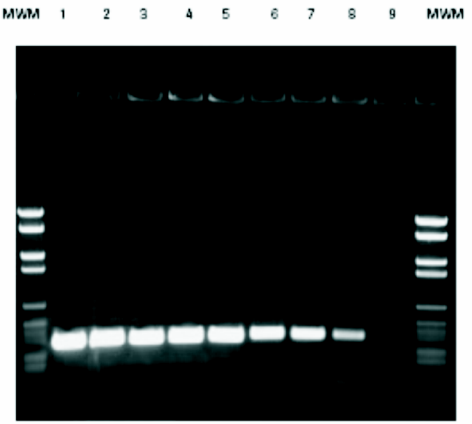

Figure 1: Amplification of 365 bp t-PA fragment down to 10 pg human genomic DNA which is equivalent to 3 gene copies for a single copy gene (3 pg is equivalent to 1 copy).

Specificity

Specificity of FastStart Taq DNA Polymerase was compared to Taq DNA polymerase by amplifying a 130 bp fragment of the human tPA gene (Figure 2).

For both enzymes, standard PCR conditions were applied (2 U/ 50 µl reaction with respective buffer conditions). 100 ng (lanes 1,7); 50 ng (lanes 2,8); 10 ng (lanes 3,9); 5 ng (lanes 4,10) controls without human genomic DNA (lanes 5,11) have been amplified (30 cycles with identical cycle program for both enzymes). Products were visualized on agarose gel. With FastStart Taq DNA Polymerase a single specific PCR product was obtained (lanes 7-10), whereas with Taq DNA polymerase unspecific PCR products and a lower sensitivity were observed (lanes 1–5).

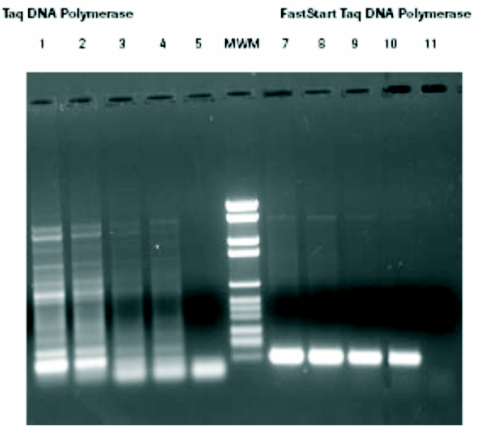

Figure 2: Highly specific PCR through "hot start" capability of FastStart Taq DNA Polymerase

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 no PCR product is formed as demonstrated on FastStart Taq DNA Polymerase alone (lane 2), Taq DNA polymerase (lane 7) or competitor A and B "hot start" Taq DNA polymerases (lane 4, 6). Competitor A´s Taq DNA polymerase combined with a special buffer (lane 5) also facilitates amplification of this target.

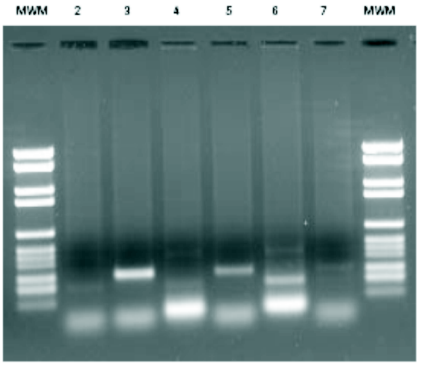

Figure 3: Amplification of a 284 bp human ApoE gene fragment (GC content 74%)
Lane 2: FastStart Taq DNA Polymerase
Lane 3: FastStart Taq DNA Polymerase + GC-RICH solution
Lane 4: Competitor A
Lane 5: Competitor A plus special buffer
Lane 6: Competitor B
Lane 7: Taq DNA Polymerase (Roche Applied Science)

	Possible Cause	Recommendation
Little or no PCR product	FastStart Taq DNA Polymerase not sufficiently activated	• Check whether PCR was started with previous activation step at +95°C for 4 min. Alternatively use 10 minutes. • Check denaturation temperature during cycles. It should be at least 30 sec. • 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 template *e.g.,* GC-rich templates	• Repeat PCR under same conditions and add GC-RICH solution (see protocol 2.3). • If performance is still not satisfying titrate GC-RICH solution (4, 6, 8, µl), reduce or increase annealing temperature, titrate Mg concentration and/ or enzyme concentration.
	Mg^{2+} concentration not optimal	Titrate Mg^{2+} concentration from 1 – 4 mM in 0.5 mM steps with buffer 3 (yellow cap).
	Primer problems due to: • design not optimized • concentration • quality or storage problems • annealing temperature too high	• If you use an established primer pair, check performance on an established PCR system (control template). • Design alternative primers. • Titrate primer concentration (0.2 – 0.5 µM). • Reduce annealing temperature.
	DNA template problems	Check quality/ concentration of template • Analyze an aliquot on a agarose gel. • Use serial dilution of template. • Make a control reaction on template with an established primer pair/PCR system. • Check/ repeat purification 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.
	Cycle conditions not optimized	• Decrease annealing temperature. • Check elongation time (1 min/ 1kb PCR fragment). • Denaturation time should not be below 30 sec. at +95°C. • Increase cycle number.

	Possible Cause	Recommendation
Multiple bands or background smear	Annealing temperature too low	Increase annealing temperature.
	Primer design or concentration not optimal	• Review primer design. • Titrate primer concentration.
	Difficult template (*e.g.,* GC-rich template)	Perform PCR with GC-RICH PCR solution
	Starting with too high concentrations of: • Mg^{2+} -ions • Template versus cycles • Enzyme	• Reduce Mg concentration. • Check template concentration by titration or by gel electrophoresis. • Use 2 U FastStart Taq per 50 µl. 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 similiar 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.
Specific problems in RT-PCR application	No product, additional bands, background smear	• The volume of cDNA template (from the RT reaction) should not exceed 10% of the final volume of the PCR reaction. • Titrate cDNA Template • Follow troubleshooting tips above.

5. Additional Information on this Product

Product Description

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 (1, 2, 3, 4) 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.

References

1 Chou,Q et al (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acid Res.* **20**:1717-1723
2 Kellogg, D.E. et al (1994) TaqStart Antibody: "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against Taq DNA polymerase. *BioTechniques* **16**:1134-1137.
3 Birch, D.E. et al (1996) Simplified hot start PCR. *Nature* **381**:445-446.
4 PCR Application Manual, Roche Applied Science, 2nd edition (1999) 2: 52-58.
5 New FastStart Taq DNA Polymerase Broadens PCR Product Line. (2001) *BIOCHEMICA* **1**: 27-29.

Quality Control

Unit Assay

1 µg M13mp9ss DNA, 0.3 µg M13 sequencing primer and 0.1 µCi [α-^{32}P] dCTP are incubated with varying amounts of units of FastStart Taq DNA Polymerase in 50 µl incubation buffer at +65°C for 60 min. The amount of incorporated dNTPs is determined.

Function test 1 (sensitivity)

Using a serial dilution of human genomic DNA, a 365 bp fragment is amplified out of tPA gene (single copy gene) under standard conditions (2 U of FastStart Taq DNA Polymerase in a 50 µl reaction). After 44 cycles, a PCR product is detectable as a single, specific band with 50 pg of starting template.

Function test 2 (GC-rich template)

A PCR assay under standard conditions is performed (2 U FastStart Taq DNA Polymerase in 50 µl reaction volume) using GC-RICH solution on 200 ng human genomic DNA with primers specific for a 284 bp fragment of the ApoE gene (74% GC content). After 35 cycles a PCR product is detectable as a single, specific band.

Endonuclease assay

1 µg lambda DNA is incubated with FastStart Taq DNA Polymerase in 50 µl test buffer at +37°C for 16 h. 25 U of enzyme show no degradation of the lambda DNA.

Exonuclease assay

5 µg of [^{3}H]-labeled calf thymus DNA is incubated with FastStart Taq DNA Polymerase in 100 µl test buffer at +65°C for 4 h. 15 U of enzyme show no release of radioactivity.

Ribonuclease assay

5 µg MS2 RNA is incubated with FastStart Taq DNA Polymerase in 50 µl test buffer at +37°C for 1 h. 25 U of enzyme show no degradation of the MS2 RNA.

Nicking activity

1 µg supercoiled pBR322 DNA is incubated with FastStart Taq DNA Polymerase in 50 µl test buffer at +37°C for 16 h. 25 U of enzyme show no relaxation of supercoiled DNA.

6. Supplementary Information

6.1 Text Conventions

To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

	Product	Pack Size	Cat. No.
DNA Purification	High Pure PCR Template Preparation Kit	100 purifications	11 796 828 001
	High Pure PCR Product Purification Kit	50 purifications 250 purifications	11 732 668 001 11 732 676 001
Kits	Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
	First Strand cDNA Synthesis Kit for RT-PCR (AMV)	1 kit	11 483 188 001
Additional Reagents	Transcriptor Reverse Transcriptase	250 U 500 U 2000 U	03 531 317 001 03 531 295 001 03 531 287 001
	GC-RICH PCR System	100 U (50 reactions)	12 140 306 001
	dATP, PCR Grade	25 µM	11 934 511 001
	dCTP, PCR Grade	25 µM	11 934 520 001
	dGTP, PCR Grade	25 µM	11 934 538 001
	dTTP, PCR Grade	25 µM	11 934 546 001
	dUTP, PCR Grade	25 µM	11 934 554 001
	Digoxigenin-11-dUTP (alkali-labile)	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 573 179 910
	Digoxigenin-11-dUTP (alkali-stable)	25 nmol (25 µl)	11 093 088 910
	Biotin-16-dUTP	50 nmol (50 µl)	11 093 070 910
	Fluorescein-12-dUTP	25 nmol (25 µl)	11 373 242 910
	PCR Nucleotide Mix	200 µl	11 581 295 001
	PCR Nucleotide Mix^{plus}	2 × 100 µl	11 888 412 001
	Water, PCR Grade	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001
	Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001

6.3 Changes to previous version

Editorial Changes

6.4 Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

6.5 Trademarks

FASTSTART and HIGH PURE are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

6.6 Regulatory Disclaimer

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

Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.

Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

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

Visit sigma-aldrich.com, to download or request copies of the following materials.

- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit sigma-aldrich.com, and select your home country to display country-specific contact information.

0715.**12165392001** ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany