Print

Expand High Fidelity^{PLUS} PCR System

Thermostable DNA polymerase blend
Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, *E.C. 2.7.7.7*

Cat. No. 03 300 242 001	125 U
Cat. No. 03 300 226 001	500 U (2 × 250 U)
Cat. No. 03 300 234 001	2500 U (10 × 250 U)

Version 09
Content version: March 2016
Store at −15 to −25°C

1. What this Product Does

Number of Tests

1 kit is designed for
- approx. 50 reactions (Cat. No. 03 300 242 001)
- approx. 200 reactions (Cat. No. 03 300 226 001)
- approx. 1000 reactions (Cat. No. 03 300 234 001)

with a final reaction volume of 50 µl each.

Contents

Vial	Label	Contents / Function A) Cat. No. 03 300 242 001 B) Cat. No. 03 300 226 001 C) Cat. No. 03 300 234 001
1	Expand High Fidelity^{PLUS} Enzyme Blend (5 U/µl)	• A) 25 µl • B) 2 × 50 µl • C) 10 × 50 µl
2	Expand High Fidelity^{PLUS} Reaction Buffer (5×) with 7.5 mM $MgCl_2$	• A) 1.25 ml • B) 2 × 1.25 ml • C) 10 × 1.25 ml
3	Expand High Fidelity^{PLUS} Reaction Buffer (5×) without $MgCl_2$	• A) 1.25 ml • B) 2 × 1.25 ml • C) 10 × 1.25 ml
4	$MgCl_2$ 25 mM Stock Solution	• A) 1 ml • B) 2 × 1 ml • C) 8 × 1 ml

Storage and Stability

If stored at −15 to −25°C, the product is stable until the expiration date printed on the label.

⚠ Always thaw and equilibrate all buffers at 37°C to 56°C before use. Vortex thoroughly. If crystals have formed, incubate at 37°C to 56°C until they are dissolved.

The kit is shipped on dry ice.

Enzyme storage buffer: 20 mM Tris-HCl, pH 8.0 (4°C), 100 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.5% Nonidet P40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v)

Application

The Expand High Fidelity^{PLUS} PCR System is a blend of Taq DNA Polymerase and a thermostable proofreading protein that lacks polymerase activity.

The Expand High Fidelity^{PLUS} PCR System is designed to amplify fragments up to 5 kb from all types of DNA with outstanding yield and fidelity. It amplifies DNA with twofold greater replicational accuracy (fidelity) than the Expand High Fidelity PCR System, and sixfold greater fidelity than Taq DNA Polymerase alone.

The proofreading protein in the Expand High Fidelity^{PLUS} was isolated and characterized by Roche. The synergy between this proofreading protein and the highly processive Taq DNA Polymerase is the key to the outstanding yield, specificity, sensitivity, and accuracy of the Expand High Fidelity^{PLUS} System.

In addition, this enzyme blend also incorporates dUTP and, in combination with Uracil-DNA Glycosylase, can be used to safeguard PCR reactions from cross contamination. Therefore, it is suited for simultaneous amplification of a large number of different targets in the same run.

These characteristics make the Expand High Fidelity^{PLUS} PCR System blend the product of choice if a larger number of targets need to be amplified at the same time with high yield in combination with improved accuracy. In addition, the product can be used for the efficient labeling of DNA fragments with radioactive or non-radioactive modified nucleotides.

Additional Equipment and Reagents Required

Additional equipment and reagents required to perform PCR reactions with Expand High Fidelity^{PLUS} PCR System, but not provided, include:
- general laboratory equipment
  - nuclease-free, aerosol-resistant pipette tips
  - pipettes with disposable, positive-displacement tips
  - sterile reaction tubes for preparing PCR mixes and dilutions
  - standard benchtop microcentrifuge
- for the PCR reaction
  - thermal block cycler
  - PCR primers
  - template DNA
  - PCR reaction vessels (thin-walled PCR tubes or plates are recommended)
  - PCR Nucleotide Mix*
  - Water, PCR-grade*
- for carry-over prevention (optional)
  - Uracil-DNA Glycosylase, heat-labile*
  - PCR Nucleotide Mix^{PLUS} *

**available from Roche Diagnostics*

Enzyme Characteristics

Volume activity	5 U/µl
Standard enzyme concentration	2.5 U per 50-µl reaction
Optimal elongation temperature	• for amplicons up to 3 kb: 72°C • for amplicons larger than 3 kb: 68°C

Mg^{2+} concentration	1.5 mM (as $MgCl_2$) when using 200 µM dNTP each. The Mg^{2+} concentration has to be optimized when dUTP is used. The optimal Mg^{2+} concentration may vary from 1.5 – 4 mM.
PCR product size	up to 5 kb
Proofreading activity	yes
Error rate [1)]	• 2-fold more accurate compared to Expand High Fidelity PCR System • 6-fold more accurate compared to Taq DNA Polymerase
PCR cloning	T/A cloning
Incorporation of modified nucleotides	accepts modified nucleotides like DIG-dUTP, Biotin-dUTP and Fluorescein-dUTP
Prevention of carryover contamination	yes

[1)] Relative fidelity determined by the lac*I* assay (1).

2. How to Use this Product

2.1 Before You Begin

General Considerations

The optimal conditions (incubation times and temperatures, concentrations of enzyme, template DNA, Mg^{2+}) are system dependend and have to be determined individually (2). In particular, the Mg^{2+} concentration and the amount of enzyme used per assay should be titrated for optimal efficiency of DNA synthesis.

As a starting point for developing your assays, use the following guidelines:

- Optimal enzyme concentration: 2.5 U/50 µl
- Optimal Mg^{2+} concentration may vary from 1.5 mM to 4 mM; in most cases a Mg^{2+} concentration of 1.5 mM will produce satisfactory results (2-3) when using 200 µM dNTP (each).
- dNTP concentration: always use balanced solutions of all four dNTPs. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. Increase concentration of Mg^{2+} when increasing the concentration of dNTPs.
- Upstream and downstream primer: it is recommended to start with 0.4 µM (final concentration) of each primer. For optimization, you may set the concentration in a range between 0.2 and 0.6 µM.

Sample Material

- Use any template DNA (*e.g.,* 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 the MagNA Pure LC Instrument or the MagNA Pure Compact Instrument together with a dedicated nucleic acid isolation kit (for automated isolation)
  – or a High Pure nucleic acid isolation kit (for manual isolation)
- Use 5–500 ng complex genomic DNA or 0.1–10 ng plasmid DNA/cDNA. Recommended starting concentrations are 250 ng genomic DNA or 1 ng plasmid DNA.

⚠ Store the template DNA either in water, PCR-grade* or 5-10 mM Tris·HCl (pH 7-8). Avoid dissolving the template in TE buffer because EDTA chelates Mg^{2+}.

2.2 Procedure

Preparation of PCR Master Mix

For each 50-µl standard reaction, prepare the following reaction mix:

🔍 See Related Procedures for prevention of carry-over contamination and incorporation of labeled nucleotides.

Step	Action
❶	• Thaw the reagents and store on ice. • Briefly vortex and centrifuge all reagents before setting up the reactions.
❷	Prepare a 10× conc. solutions of the PCR primers. 🔍 If you want to achieve for each primer *e.g.,* a final concentration of 0.4 µM, the 10× conc. solution should contain 4 µM of each primer.
❸	For each 50-µl reaction, add the components in the order listed below to a sterile reaction tube on ice:

Reagent	Volume	Final Concentration
water, PCR-grade	add up to 50 µl	
Expand HiFi^{PLUS} Reaction Buffer, 5× (vial 2)	10 µl	1×
PCR Nucleotide Mix, PCR-grade (10 mM)	1 µl	0.2 mM
upstream primer (10× conc.)	5 µl	0.4 µM
downstream primer (10× conc.)	5 µl	0.4 µM
template DNA	variable	5–500 ng (genomic DNA) 100 pg–10 ng (plasmid DNA)
Expand HiFi^{PLUS} Enzyme Blend (5 U/µl) (vial 1)	0.5 µl	2.5 U
Total Volume	**50 µl**	

🔍 [a)] To prepare the PCR mix for more than one reaction, multiply the amounts in the "Volume" column by z, where z = the number of reactions to be run + one additional reaction.

❹	Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube. 🔍 Overlay the reaction carefully with mineral oil if required by your type of thermal block cycler. ⚠ Start thermal cycling immediately. Do not store complete reaction mixes on ice.

2.3 PCR

🔍 The thermal profiles were developed for the Applied Biosystems GeneAmp PCR System 9600. Other thermal cyclers may require different profiles.

Thermal profile A: fixed elongation time

Program	Cycles	Time	Temp
Initial denaturation	1	2 min	+94°C [a)]
Denaturation Annealing Elongation	25-35	10 – 30 s 30 s 30 s – 4 min [d)]	+94°C [a)] +55 to +68°C [b)] +68 to +72°C [c)]
Final elongation	1	7 min	+68 to +72°C
Cooling		indefinitely	+4°C

Thermal profile B: gradually increasing extension time

🔍 This procedure ensures a higher yield of amplification products.

Program	Cycles	Time	Temp
Initial denaturation	1×	2 min	+94°C [a)]
Denaturation Annealing Elongation	10	10 – 30 s 30 s 30 s – 4 min [d)]	+94°C [a)] +55 to +68°C [b)] +72°C

Denaturation		15 – 30 s	+92 to +94°C [a]
Annealing	15-20	30 – 60 s	+55 to + 68°C [b]
Elongation		30 s – 4 min [d] + 10 s cycle elongation for each succ. cycle [e]	+68 to +72°C [c]
Final elongation	1×	7 min	+68 to +72°C [c]
Cooling		indefinitely	+4°C

[a] Optimal denaturation temperature and time depends upon the GC content of the template.
[b] Optimal annealing temperature depends on the melting temperature of the primers and the system used.
[c] For PCR products up to 3 kb elongation temperature should be +72°C; for PCR products larger than 3 kb elongation temperature should be +68°C.
[d] Elongation time depends upon length of the product to be amplified (~1 min per kb)
[e] For example, cycle no. 11 is 10 s longer than cycle 10, cycle no. 12 is 20 s longer than cycle 10, cycle no. 13 is 30 s longer than cycle 10, etc.

After cycling, if the samples are not used immediately, store them frozen for later use.

For best results, do the following:
- Check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker.
- Purify the PCR product with the High Pure PCR Product Purification Kit* (*e.g.,* before performing nested PCR).

2.4 Related Procedures

Prevention of Carry-Over Contamination

Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination in PCR. This carry-over prevention technique involves incorporating deoxyuridine triphosphate into amplification products, then pretreating later PCR mixtures with UNG. If a dUTP-containing contaminant is present in the later PCRs, it will be cleaved by a combination of the UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template. Since your target DNA template contains thymidine rather than uridine, it is not affected by this procedure.

Adapt the protocol under "Preparation of PCR Master Mix" on page 2 as follows:

- Use Expand High Fidelity^{PLUS} Reaction Buffer (5×) without $MgCl_2$ (vial 3) and $MgCl_2$, 25 mM stock solution (vial 4) in concentrations ranging from 1.5 mM up to 4 mM final.
- Use PCR Nucleotide Mix^{PLUS}* which contains 200 mM dATP, dCTP, dGTP and 600 mM dUTP (final conc.)

Proceed as described below to prevent carry-over contamination using heat-labile UNG:

❶	Per 50 µl final reaction volume, add 2 µl heat-labile UNG to the RT-PCR mix.
❷	Add DNA template and incubate the reaction mixture for 10 min at RT to destroy any contaminating template.
❸	Inactivate heat-labile UNG by performing the initial denaturation step at +94°C for 2 min.

Incorporation of Non-radioactive Modified Nucleotides

You can use the Expand High Fidelity^{PLUS} PCR System to incoporate non-radioactive modified nucleotides (*e.g.,* DIG-dUTP*, Biotin-dUTP*, Fluorescein-dUTP*, or Rhodamine-dUTP*) into PCR products.

To do this, exchange the PCR Nucleotide Mix by the following nucleotide mixes:

- 10× DIG-dUTP nucleotide mix: 0.7 mM DIG-dUTP, 1.3 mM dTTP, 2 mM dCTP, 2 mM dATP and 2 mM dGTP. A final $MgCl_2$ concentration of 3 mM can be used, however, it is recommended to optimize the concentration for each new reaction.
- 10× Biotin-16-dUTP nucleotide mix: 0.7 mM Biotin-16-dUTP, 1.3 mM dTTP, 2 mM dCTP, 2 mM dATP and 2 mM dGTP. A final $MgCl_2$ concentration of 1.5 mM is recommended.
- 10× Fluorescein-16-dUTP nucleotide mix: 0.5 mM Fluorescein-16-dUTP, 1.5 mM dTTP, 2 mM dCTP, 2 mM dATP and 2 mM dGTP. A final $MgCl_2$ concentration of 1.5 mM is recommended.

3. Troubleshooting

Problem	Possible Cause	Recommendation
Little or no PCR product	Pipetting errors	Check all concentrations and storage conditions of reagents.
	Difficult template (*e.g.,* GC-rich)	• Use the GC-rich PCR System*. • Add DMSO (at 8%) and titrate enzyme concentration down to 0.5 U/reaction.
	DNA template problems	Check quality and concentration of template: • Analyze an aliquot of the template on an agarose gel to check for possible degradation. • Perform a control reaction on template using an established primer pair. • Check or repeat purification of template.
	Enzyme concentration too low	Increase enzyme concentration (in 0.5 U steps) to 2 U/50 µl reaction.
	$MgCl_2$ concentration too low	Increase the $MgCl_2$ concentration in steps of 0.25 mM.
	Cycle conditions not optimal	• Decrease annealing temperature. • Increase cycle number. • Make sure the final elongation step is carried out.
Primer problems	Primer design	Design alternative primers
	Primer concentration	• Make sure both primers have the same concentration. • Titrate primer concentration.
	Annealing temperature too high	• Reduce annealing temperature. • Determine the optimal annealing temperature by touch-down PCR.
	Primer specificity	• Perform nested PCR.
	Quality or storage	• If you use an established primer pair, check performance on a control template. • Make sure the primers are not degraded. • Always store primers at –15 to –25°C.
Multiple bands or background smear	Annealing temperature too low	Increase annealing temperature according to the primer length.
	Primer design or concentration	• Check primer design. • Titrate primer concentration (0.1 - 0.6 µM) • Make sure both primers have the same concentration. • Perform nested PCR.
	Difficult template (*e.g.,* GC-rich)	• Use the GC-rich PCR System*. • Add DMSO (at 8%) and titrate enzyme concentration down to 0.5 U/reaction.
	DNA template	Use serial dilutions of template.

Problem	Possible Cause	Recommendation
PCR products in negative control experiments	Carryover contamination	• Exchange all reagents, especially water. • Use aerosol resistant tips. • Set up PCR reactions in an area separated from that used for PCR product analysis. • Use dUTP (600 µM) instead of dTTP in combination with heat-labile UNG* (1 U/50-µl reaction) and increase Mg^{2+} concentration to a maximum of 4 mM.
Specific problems in RT-PCR application	No product, additional bands, background smear	• The volume of unpurified cDNA template should not exceed 1/10th of the PCR reaction. • Increase $MgCl_2$ by titration in steps of 0.25 mM. • Follow the troubleshooting hints above.

4. Additional Information on this Product

Quality Control

Each lot of Expand High Fidelity^{PLUS} PCR System is function tested in PCR using human genomic DNA and (1) primers specific for the human erythropoetin gene to yield a 1.8 kb PCR product and (2) primers specific for the human tPA gene to yield a 4.8 kb PCR product.

References

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Product Citations

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5. Supplementary Information

5.1 Conventions

Text Conventions

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

Text Convention	Usage
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.

Abbreviations

In this Instruction Manual the following abbreviations are used:

Abbreviation	Meaning
HiFi	High Fidelity
UNG	Uracil-N DNA-glycosylase

5.2 Ordering Information

Product	Pack Size	Cat No.
Expand High Fidelity^{PLUS} PCR System, dNTPack	125 U 500 U (2 × 250 U)	04 743 725 001 04 743 733 001
GC-Rich PCR System, dNTPack	100 U	04 743 784 001
PCR Nucleotide Mix	200 µl 10 × 200 µl	11 581 295 001 11 814 362 001
PCR Nucleotide Mix^{PLUS}	200 µl 10 × 200 µl	11 581 295 001 11 814 362 001
Uracil-DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001
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
High Pure PCR Product Purification Kit	Kit for 50 purifications Kit for 250 purifications	11 732 668 001 11 732 676 001
Biotin-16-dUPT	50 nmol (50 µl)	11 093 070 910
Digoxigenin-11-dUTP, alkali-labile	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 573 179 910
Fluorescein-12-dUTP	25 nmol (25 µl)	11 373 242 910
Tetramethyl-Rhodamine-5-dUTP	25 nmol (25 µl)	11 534 378 910

5.3 Changes to previous version

- Editorial Changes

Contact and Support

To ask questions, solve problems, suggest enhancements and 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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