Expand High Fidelity PCR System

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

Cat. No. 11 732 641 001	100 U
Cat. No. 11 732 650 001	500 U (2 × 250 U)
Cat. No. 11 759 078 001	2,500 U (10 × 250 U)

Version 21
Content version: March 2016
Store at −15 to −25°C

1. What this Product Does

Number of Tests

The kit is designed for

- approx. 40 reactions (Cat. No. 11 732 641 001)
- approx. 200 reactions (Cat. No. 11 732 650 001)
- approx. 1000 reactions (Cat. No. 11 759 078 001)

with a final reaction volume of 50 µl each.

Kit Contents

Vial	Label	Contents
1	Expand High Fidelity Enzyme mix	• 30 µl (100 U pack size) • 2 × 75 µl (500 U pack size) • 10 × 75 µl (2,500 U pack size) Enzyme storage buffer: 20 mM Tris-HCl, pH 7.5 (+25°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)
2	Expand High Fidelity Buffer (10×) with 15 mM $MgCl_2$	• 1 ml (100 U pack size) • 2 × 1 ml (500 U pack size) • 10 × 1 ml (2,500 U pack size)
3	Expand High Fidelity Buffer (10×) without $MgCl_2$	• 1 ml (100 U pack size) • 1 ml (500 U pack size) • 10 × 1 ml (2,500 U pack size)
4	$MgCl_2$ 25 mM Stock Solution	• 1 ml (100 U pack size) • 1 ml (500 U pack size) • 10 × 1 ml (2,500 U pack size)

Storage and Stability

Store the kit at −15 to −25°C. When properly stored, the kit 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.

Additional Equipment and Reagents Required

- dNTP Mix*, Primer; template DNA; Water, PCR Grade*
- Thermal block cycler (*e.g.,* Applied Biosystems GeneAmp PCR System 9600)
- 0.2 ml thin-walled PCR tubes
- Sterile reaction (Eppendorf) tubes for preparing master mixes and dilutions

Applications

PCR and DNA labeling reactions

Expand High Fidelity PCR System is especially optimized to efficiently amplify DNA fragments up to 5 kb. PCR is possible up to 9 kb with yield diminishing as DNA fragment length increases.

For the generation of longer PCR products, the Expand Long Template PCR System, which is optimized for the amplification of DNA fragments ranging from 3 kb to 27 kb in length, is recommended.

Expand High Fidelity PCR System is composed of a special enzyme mix containing thermostable Taq DNA polymerase and Tgo DNA polymerase, a thermostable DNA polymerase with proofreading activity. This powerful polymerase mixture is designed to generate PCR products of high yield, high fidelity and high specificity from all types of DNA (1).

Due to the inherent 3´-5´ exonuclease or "proofreading" activity of Tgo DNA polymerase, the fidelity of DNA synthesis with Expand High Fidelity PCR System shows a threefold increase compared to Taq DNA polymerase.

Enzyme Properties

Volume activity	3.5 U/µl
Error rate1	threefold more accurate compared to Taq DNA Polymerase
Standard enzyme concentration	2.6 U (0.75 µl) per 50 µl reaction
Optimal enzyme concentration	varies from 0.5 – 5 U per 50 µl reaction
Optimal elongation temperature	72°C. For PCR products >3 kb the optimal elongation temperature is 68°C.
Optimal Mg^{2+} concentration	varies from 1.5 – 4 mM (as $MgCl_2$)
Standard Mg^{2+} concentration	1.5 mM (as $MgCl_2$) when using 200 µM dNTP each.
PCR product size	up to 5 kb
PCR Cloning	T/A cloning
Repair of mismatched primers at 3´ end	yes, due to the 3´-5´ exonuclease activity of the proofreading polymerase
Incorporation of modified nucleotides	accepts modified nucleotides like DIG-dUTP, Biotin-dUTP and Fluorescein-dUTP**
Prevention of carry-over prevention	no***

1) Relative fidelity determined by the lacI assay (1).

** For generating probes for Southern analysis the concentration of modified dUTP should be 50 µM (with 150 µM dTTP). When using fluorescein-dUTP the $MgCl_2$ concentration should be increased to 4 mM. For ELISA based detection systems a concentration of 10 µM modified dUTP is sufficient

*** Unlabeled dUTP (instead of dTTP) is a poor substrate for the Expand enzyme mix. Therefore it is not recommended to use the Expand enzyme mix in combination with UNG carry over prevention.

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+}) depend on the system used and have to be determined individually. In particular, the Mg^{2+} concentration and the amount of Expand enzyme mix 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: 0.5 – 5 U/50 µl. The recommended starting concentration is 2.6 U (0.75 µl).
- Optimal Mg^{2+} concentration can vary from 1.5 - 4 mM. The recommended starting concentration is 1.5 mM when using 200 µM dNTP (each).
- dNTP concentration: always use balanced solutions of all four dNTP. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. Increase concentrations of Mg^{2+} when increasing the concentration of dNTP.
- The optimal buffer for the template DNA is either simply PCR grade water or 5 - 10 mM Tris (pH 7 - 8). Avoid dissolving the template in TE buffer because EDTA chelates Mg^{2+}.
- Usually it is not necessary to add additives. Nevertheless in some cases improvements can be achieved by using up to 100 mg/ml bovine serum albumin (BSA), 0.1% Tween 20 (v/v) or 1 - 2% DMSO.

Sample Material

Template DNA, *e.g.,* human genomic DNA*

⚠ The quality of the template has a tremendous effect on the success of the PCR.

2.2 Preparation of the Reaction Mixes

For a larger number of reactions, we recommend that you prepare two reaction mixes. This circumvents the need of "Hot Start" and avoids that the 3´-5´ exonuclease activity of the proofreading polymerase partially degrades primers and template during the reaction set-up.

It is also recommended to prepare a Master Mix for setting up multiple reactions. The Master Mix typically contains all of the components needed for all PCR tests to be performed at a volume 10% greater than that required for the total number of PCR assays.

❶ Briefly vortex and centrifuge all reagents before starting.

❷ • Prepare two mixes in a sterile microfuge tubes (on ice):
• **Mix 1** (for one reaction):

Reagent	Volume	Final conc.
sterile double-dist. water	add up to 25 µl	
Deoxynucleotide mix, 10 mM of each dNTP)	1 µl	200 µM of each dNTP
Upstream primer	variable	300 nM
Downstream primer	variable	300 nM
Template DNA	variable	0.1 - 250 ng[a]
Final volume	**25 µl**	

Mix 2 (for one reaction):

Reagent	Volume	Final conc.
sterile double-dist. water	19.25 µl	
Expand High Fidelity buffer, 10× conc. with 15 mM $MgCl_2$	5 µl	1× (1.5 mM $MgCl_2$)
Expand High Fidelity enzyme mix	0.75 µl	2.6 U/reaction
Final volume	**25 µl**	

🔍 When titrating the Mg^{2+} concentration use the Expand High Fidelity buffer, 10× conc. without $MgCl_2$ and the $MgCl_2$ stock solution (25 mM).

❸ • Combine Mix 1 and Mix 2 in a thin-walled PCR tube (on ice).
• Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect sample at the bottom of the tube.

🔍 Overlay the reaction carefully with mineral oil if required by your type of thermal cycler.

[a] *e.g.,* human genomic DNA template: 10 ng - 250 ng; plasmid DNA template: 0.1 ng - 15 ng.

2.3 Thermal Cycling

Place samples in the thermal block cycler, and start cycling using the thermal profile below. The gradually increasing extension time ensures a higher yield of amplification products.

🔍 The elongation step should be performed at **+68°C** when PCR products longer than 3 kb are amplified.

	Temperature	Time	Cycles
Initial Denaturation	94°C	2 min	1×
Denaturation Annealing Elongation	94°C 45 – 65°C[b] 68 or 72°C[c]	15 s 30 s 45 s – 8 min[d]	10×
Denaturation Annealing Elongation	94°C 45 – 68°C[b] 72°C[c]	15 s 30 s 45 s – 8 min[d]+ 5 s cycle elongation for each successive cycle[e]	15 – 20×
Final Elongation	72°C[c]	7 min	1×
Cooling	4°C	unlimited time	

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 on fragment length: 45 s for up to 0.75 kb, 1 min for 1.5 kb, 2 min for 3 kb, 4 min for 6 kb, 8 min for 10 kb.

e) For example, cycle no. 11 is 5 s longer than cycle 10, cycle no. 12 is 10 s longer than cycle 10, cycle no. 13 is 15 s longer than cycle 10, etc.

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

3. Troubleshooting

	Possible Cause	Recommendation
Little or no PCR product	Difficult template *e.g.,* GC-rich templates	• Perform initial denaturation step at 95°C for 3 - 5 min. • Use the GC-RICH PCR System*.
	Poor DNA template quality	Check quality and concentration of template: • Analyze an aliquot on an agarose gel to check for possible degradation. • Include a control reaction using a known template under established PCR conditions. • Check or repeat purification of template.
	Enzyme concentration too low	Increase the amount of enzyme mix in 0.5 U steps.
	$MgCl_2$ concentration too low	Increase the $MgCl_2$ concentration in 0.25 mM steps. (Minimum concentration is 1.5 mM $MgCl_2$.)
	Cycle conditions not optimal	• Reduce annealing temperature. • Increase number of cycles. • Be sure to perform the final elongation step.
	Primer design not optimal	Design alternative primers.
	Primer concentration not optimal	• Both primers must have the same concentration. • Titrate primer concentration (0.2 – 0.6 µM).
	Annealing temperature too high	• Reduce annealing temperature. (Minimum annealing temperature is 45°C.) • Determine the optimal annealing temperature by touch-down PCR.
	Primer quality or storage problems	• If you are using an established primer pair, check their performance under established PCR conditions (with a control template). • Make sure primers are not degraded. • Always store primers at −15 to −25°C.
	Formation of primer dimers	Use two reaction mixes according to the protocol above.
Multiple bands or background smear	Annealing temperature too low	Increase annealing temperature according to primer length.
	Primer design or concentration not optimal	• Review primer design. • Titrate primer concentration (0.2 – 0.6 µM). • Both primers must be present in the reaction at the same concentration. • Perform nested PCR with nested primers.
	Difficult template *e.g.,* GC- rich template	Use the GC-RICH PCR System (see Ordering Information).
	DNA template problems	Use serial dilutions of template.
PCR products in negative control experiments	Carry-over contamination	• Exchange all reagents, especially water. • Use aerosol-resistant pipette tips. • Set up PCR reactions in an area separate from that used for PCR product analysis.
Specific problems in RT-PCR	No product, additional bands, background smear	• The volume of cDNA template (RT-reaction) should not exceed 10% of the final concentration of the PCR reaction. • Follow trouble shooting above. • Increase $MgCl_2$ by titration in steps of 0.25 mM.

4. Additional Information on this Product

4.1 How this Product Works

References

1 Barnes, W.M. (1994) *Proc. Natl. Acad. Sci.* USA **91**, 2216–2220.
2 Frey, B. & Suppmann, B. (1995) *Biochemica* **2**, 8-9.

For general information on PCR, please see the following (available on our website):

3 PCR Applications Manual
4 PCR Product Family Flyer
5 Lab FAQS “Find a Quick Solution“

Quality Control

Each lot of Expand High Fidelity PCR System is function tested in PCR. Routinely Expand High Fidelity PCR is performed using human genomic DNA and specific primers for the tPA gene to get a 4.8 kb PCR product.

5. Supplementary Information

5.1 Symbols

Symbols are used in this document 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.

5.2 Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Convention	Use
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 Applied Science.

5.3 Ordering Information

	Product	Pack Size	Cat. No.
Standard PCR	Taq DNA Polymerase	100 U	11 146 165 001
		500 U	11 146 173 001
		4 × 250 U	11 418 432 001
		10 × 250 U	11 596 594 001
		20 × 250 U	11 435 094 001
	PCR Core Kit[PLUS]	1 kit	11 585 541 001
	PCR Core Kit	1 kit	11 578 553 001
	PCR Master	1 kit	11 636 103 001
Maximum specificity	FastStart Taq DNA Polymerase (Hot start)	100 U	12 032 902 001
		500 U	12 032 929 001
		4 × 250 U	12 032 937 001
		10 × 250 U	12 032 945 001
		20 × 250 U	12 032 953 001
	FastStart High Fidelity PCR System (Hot start)	125 U	03 553 426 001
		2 × 250 U	03 553 400 001
		10 × 250 U	03 553 361 001
High fidelity PCR	Pwo SuperYield DNA Polymerase	100 U	04 340 868 001
		2 × 250 U	04 340 850 001
	Pwo Master	1 kit	03 789 403 001
	Pwo DNA Polymerase	100 U	11 644 947 001
		2 × 250 U	11 644 955 001
	Expand High Fidelity PCR System	100 U	11 732 641 001
		2 × 250 U	11 732 650 001
		10 × 250 U	11 759 078 001
	High Fidelity PCR Master	1 kit	12 140 314 001
	Expand High Fidelity[PLUS] PCR System	125 U	03 300 242 001
		2 × 250 U	03 300 226 001
		10 × 250 U	03 300 234 001
Long template PCR	Expand Long Template PCR System	150 U	11 681 834 001
		2 × 360 U	11 681 842 001
		10 × 360 U	11 759 060 001
	Expand 20 kb[PLUS] PCR System	200 U	11 811 002 001
Difficult templates & challenging assays	FastStart High Fidelity PCR System (Hot start)	125 U	03 553 426 001
		2 × 250 U	03 553 400 001
		10 × 250 U	03 553 361 001
	GC-RICH PCR System	100 U	12 140 306 001
Ready-to-use mixes of all 4 nucleotides	PCR Nucleotide Mix PCR Grade, Na-salt sol.	200 µl	11 581 295 001
		2,000 µl	11 814 362 001
DNA purification	High Pure PCR Template Preparation Kit	100 purifications	11 796 828 001
	High Pure PCR Product Purification Kit	50 purifications	11 732 668 001
		250 purifications	11 732 676 001
Additional reagents	Water, PCR Grade	25 ml (25 vials of 1 ml)	03 315 932 001
		25 ml (1 vial of 25 ml)	03 315 959 001
		100 ml (4 vials of 25 ml)	03 315 843 001
	Biotin-16-dUTP	50 nmol (50 µl)	11 093 070 910
	PCR Cloning Kit (blunt end)	35 cloning and 5 control reactions	11 939 645 001
	Human Genomic DNA	100 µg (500 µl)	11 691 112 001
	Human t-PA Control Primer Set	1 set	11 691 104 001
	Bovine Serum Albumin	20 mg (1 ml)	10 711 454 001

Changes to Previous Version

Editorial changes

Trademarks

Regulatory Disclaimer

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

Disclaimer of License

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

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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