For general laboratory use. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

Expand Long Template PCR System

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

Cat. No. 11 681 834 001 150 U
Cat. No. 11 681 842 001 720 U (2× 360 U)
Cat. No. 11 759 060 001 3 600 U (10× 360 U)

Version September 2005
Store the kit at –15 to –25°C

1. What this Product Does

Number of Tests

The kit is designed for

- approx. 38 reactions (Cat. No. 11 681 834 001)
- approx. 190 reactions (Cat. No. 11 681 842 001)
- approx. 950 reactions (Cat. No. 11 759 060 001)

with a final reaction volume of 50 µl each.

Kit Contents

Vial	Label	Contents
1	Expand Long Template Enzyme mix	• 30 µl (150 U pack size) • 2 × 72 µl (720 U pack size) • 10 × 72 µl (3600 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 Long Template buffer **1**	10× conc. with 17.5 mM $MgCl_2$ • 1 ml (150 U pack size) • 2 × 1 ml (720 U pack size) • 10 × 1 ml (3 600 U pack size)
3	Expand Long Template buffer **2**	10× conc. with 27.5 mM $MgCl_2$ • 1 ml (150 U pack size) • 2 × 1 ml (720 U pack size) • 10 × 1 ml (3 600 U pack size)
4	Expand Long Template buffer **3**	10× conc. with 27.5 mM $MgCl_2$ and detergents • 1 ml (150 U pack size) • 2 × 1 ml (720 U pack size) • 10 × 1 ml (3 600 U pack size)

Storage and Stability

Store the kit at –15 to –25°C. When properly stored, the kit is stable through 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.

Once the kit is opened, store the kit components as described in the following table:

Vial	Label	Storage
1	Expand Long Template Enzyme mix	• Aliquot and store at –15 to –25°C. • Avoid repeated freezing and thawing!
2	Expand Long Template buffer **1**	Store at –15 to –25°C.
3	Expand Long Template buffer **2**	
4	Expand Long Template buffer **3**	

** available from Roche Applied Science*

Additional Equipment and Reagents Required

- 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

Application

Expand Long Template PCR System is a unique enzyme mix that contains thermostable Taq DNA polymerase and Tgo DNA polymerase (1), a thermostable DNA polymerase with proofreading activity. This powerful polymerase mixture produces a high yield of PCR product from genomic DNA. Expand Long Template PCR System is optimized to efficiently amplify large genomic DNA fragments around 20 kb long.

Due to the inherent 3'-5' exonuclease ("proofreading") activity of Tgo DNA polymerase, Expand Long Template PCR System copies DNA threefold more accurately than Taq DNA polymerase.

Enzyme Properties

Volume activity	5 U/µl
Error rate[1]	3-fold more accurate compared to Taq DNA Polymerase
Optimal enyzme concentration	varies from 0.5 to 5.0 U per 50 µl reaction
Standard enzyme concentration	3.75 U per 50 µl reaction
Optimal elongation temperature	68°C
Standard Mg^{2+} concentration	1.75 mM (as $MgCl_2$) when using 350 µM of each dNTP and 2.75 mM (as $MgCl_2$) when using 500 µM of each dNTP
PCR product size	around 20 kb
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
Incorporation of dUTP	No

[1)] Relative fidelity determined by the lacI assay (2).

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+} concentration) depend on the system used and must be determined for each system. In particular, to ensure optimal reaction efficiency, you should titrate the Mg^{2+} concentration and the amount of enzyme used per assay. If you are developing a new assay, you should test all three amplification systems to find the one that gives the best results.

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

- Optimal enzyme concentration: 0.5 to 5.0 U/µl. The recommended starting concentration is 3.75 U (0.75 µl).
- The quality of the template has a tremendous effect on the success of the PCR.

  Human genomic DNA and a human tPA Control Primer Set – especially tested with the Expand Long Template PCR System – are available from Roche Applied Science.
- dNTP concentration: Always use balanced concentrations of all four dNTPs. The final concentration of each dNTP should be between 350 and 500 µM.

  If you increase the concentration of dNTP, also increase the Mg^{2+} concentration.
- The optimal buffer for dilution of the template DNA is either sterile double-distilled water or 5 to 10 mM Tris (pH 7-8). Avoid dissolving the template in TE buffer because EDTA chelates Mg^{2+}.
- Reactions do not usually require additives. Nevertheless, in some cases you can improve yield by adding up to 100 µg/ml bovine serum albumin (BSA), 0.1% Tween 20 (v/v) or 1 to 2% DMSO.
- Use 0.2 ml thin-walled PCR tubes.
- Keep denaturation steps as short as possible and denaturation temperature as low as possible.

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 Setting up the Experiment

Preparation of Reaction Mixes

- Always thaw and equilibrate all buffers at 37°C to 56°C before use. Vortex thoroughly.
- Briefly vortex and centrifuge all reagents before starting.
- Set up the reaction components in a sterile microfuge tube (on ice).
- After pipetting the last reaction component, start the reactions immediately. Do not store complete reaction mixes on ice.
- If the initial reaction produces too many primer dimers, try using two separate master mixes. (Master Mix 1 contains dNTPs, primers, and template DNA; Master Mix 2 contains buffer and enzyme. Final volume of each mix: 25 µl. For final conc. of each component, see below.) Add these two to the tube on ice, then just before starting the reaction, vortex the tubes to produce a homogeneous reaction mix.

If the amplification system recommended for a given fragment length does not give satisfactory amplification, repeat the experiment using one of the other two possible amplification systems.

Amplification of human gen. DNA	**0.5 – 9 kb System 1**		**9 – 12 kb System 2**		**> 12 kb System 3**	
Components	**Vol.**	**Final conc.**	**Vol.**	**Final conc.**	**Vol.**	**Final conc.**
add sterile double dist. H_20	50 µl		50 µl		50 µl	
dATP*10 mM	1.75 µl	350 µM	2.5 µl	500 µM	2.5 µl	500 µM
dCTP*10 mM	1.75 µl	350 µM	2.5 µl	500 µM	2.5 µl	500 µM
dGTP*10 mM	1.75 µl	350 µM	2.5 µl	500 µM	2.5 µl	500 µM
dTTP*10 mM	1.75 µl	350 µM	2.5 µl	500 µM	2.5 µl	500 µM
Downstream primer	x µl	300 nM	x µl	300 nM	x µl	300 nM
Upstream primer	y µl	300 nM	y µl	300 nM	y µl	300 nM
Components	**Vol.**	**Final conc.**	**Vol.**	**Final conc.**	**Vol.**	**Final conc.**
10x PCR buffer with $MgCl_2$	5 µl buffer 1		5 µl buffer 2		5 µl buffer 3	
template DNA*	z µl	(1.75 mM) up to 500 ng genom. DNA	z µl	(2.75 mM) up to 500 ng genom. DNA	z µl	(2.75 mM) up to 500 ng genom. DNA
Expand Long Template enzyme mix	0.75 µl		0.75 µl		0.75 µl	

* Instead of using single dNTP solutions, you may use the PCR Grade Nucleotide Mix.

Thermal Cycling

Place samples in the thermal block cycler, and cycle according to the thermal profile below. Gradually increasing extension time ensures a higher yield of amplification products.

	Temperature	**Time**	**Cycles**
Initial Denaturation	92 to 94°C	2 min	1
Denaturation Annealing Elongation	92 to 94°C 45 to 65°C[a] 68°C	10 s 30 s 45 s – 30 min[b]	10
Denaturation Annealing Elongation	92 to 94°C 45 to 65°C[a] 68°C	15 s 30 s 45 s – 30 min[b]+ 20 s cycle elongation for each successive cycle[c]	15 – 25
Final Elongation	68°C	7 min	1
Cooling	4°C	unlimited time	

[a] Optimal annealing temperature depends on the melting temperature of the primers and the system used.

[b] Elongation time depends on fragment length: Use 2 min for up to 3 kb, 4 min for 6 kb, 8 min for 10 kb, 15 min for 20 kb, 20 min for 30 kb.

[c] For example, cycle no. 11 is 20 s longer than cycle 10, cycle no. 12 is 40 s longer than cycle 10, cycle no. 13 is 60 s longer than cycle 10, etc.

The thermal profile above was developed for the Applied Biosystems GeneAmp PCR System 9600. Other thermal block cyclers may require a different profile. Long range PCR in general is sensitive to even minute differences between ramping or heat transfer rates of different thermal block cyclers. Therefore, always develop and run your Expand Long Template PCR experiment on the same thermal block cycler. If you switch to a different thermal block cycler, adjust the reaction conditions and thermal profile.

3. Troubleshooting

	Possible Cause	Recommendation
Little or no PCR product	Difficult template	Try amplification system 2 or 3 first, even if you have short amplicons.
	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 1.75 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 be present in the reaction at the same concentration. • Titrate primer concentration (0.3 – 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.
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 (0.3 – 0.6 µM). • Both primers must be present in the reaction at the same concentration. • Perform nested PCR with nested primers. • Primers should have similar melting temperatures.
	DNA template problems	Use serial dilutions of template.
PCR products in negative control experiments	Carry-over contamination	• Replace 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 (from RT reaction) should not exceed 10% of the final PCR reaction volume. • Increase $MgCl_2$ in 0.25 mM steps. • Follow troubleshooting suggestions above.

4. Additional Information on this Product

References

1 Hopfner, K.P. et al. (1999) *Proc. Natl Acad. Sci.* **96**, 3600-3605.
2 Frey, B. & Suppmann, B. (1995) *Biochemica* **2,** 8-9.
3 Lin, Z. et al (2000) Polymorphisms of human SP-A, SP-B, and SP-D genes: association of SP-B Thr131Ile with ARDS. *Clinical Genetics* **58**, 181.
4 van Beilen; J.B. et al (2001) Analysis of Pseudomonas putida alkane-degradation gene clusters and flanking insertion sequences: evolution and regulation of the alk genes. *Microbiology* **147,** 1621-1630.

For general information on PCR, please see the following (available on our website):
5 PCR Special Interest Site: http://www.roche-applied-science.com/PCR
6 PCR Applications Manual, 2nd Edition
7 PCR Product Family Flyer
8 Lab FAQS "Find a Quick Solution"

Quality Control

Each lot of Expand Long Template PCR System is function tested in PCR. Routinely, Expand Long Template PCR is used to amplify a human genomic DNA with primers that are specific for 9 kb, 12 kb and 15 kb fragments. The enzyme mix is tested for the absence of any contaminating activities, including endo- or exonucleases and nicking activity.

5. Supplementary Information

5.1 Symbols

In this pack insert, 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.

5.2 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page
www.roche-applied-science.com/PCR

	Product	Pack Size	Cat. No.
Standard PCR	Taq DNA Polymerase	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 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
	Expand High Fidelity-^{PLUS} PCR System	125 U 2 × 250 U 10 × 250 U	03 300 242 001 03 300 226 001 03 300 234 001
	Expand High Fidelity PCR System	100 U 2 × 250 U 10 × 250 U	11 732 641 001 11 732 650 001 11 759 078 001
	High Fidelity PCR Master	1 kit	12 140 314 001
Maximum specificity	FastStart Taq DNA Polymerase (Hot start)	100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	FastStart High Fidelity PCR System (Hot start)	125 U 2 × 250 U 10 × 250 U	03 553 426 001 03 553 400 001 03 553 361 001

	Product	Pack Size	Cat. No.
High fidelity PCR	Pwo SuperYield DNA Polymerase	100 U 2 ×250 U	04 340 868 001 04 340 850 001
	Pwo Master	1 kit	03 789 403 001
	Pwo DNA Polymerase	100 U 2 ×250 U	11 644 947 001 11 644 955 001
	Expand High Fidelity PCR System	100 U 2 × 250 U 10 × 250 U	11 732 641 001 11 732 650 001 11 759 078 001
	High Fidelity PCR Master	1 kit	12 140 314 001
	FastStart High Fidelity PCR System (Hot start)	125 U 2 ×250 U 10 ×250 U	03 553 426 001 03 553 400 001 03 553 361 001
	Expand High Fidelity-PLUS PCR System	125 U 2 × 250 U 10 × 250 U	03 300 242 001 03 300 226 001 03 300 234 001
Long template PCR	Expand Long Template PCR System	150 U 2 × 360 U 10 × 360 U	11 681 834 001 11 681 842 001 11 759 060 001
	Expand 20 kbPLUS PCR System	200 U	11 811 002 001
Difficult templates & challenging assays	FastStart Taq DNA Polymerase (Hot start)	50 U 100 U 500 U 4 × 250 U 10 × 250 U 20 × 250 U	12 158 264 001 12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
	FastStart High Fidelity PCR System (Hot start)	125 U 2 × 250 U 10 × 250 U	03 553 426 001 03 553 400 001 03 553 361 001
	GC-RICH PCR System	100 U	12 140 306 001
Nucleotide sets containing all 4 dNTPs in separate vials	Deoxynucleoside Triphosphate Set, PCR Grade, Na-salt sol	4 × 250 µl 4 × 1,250 µl	11 969 064 001 03 622 614 001
	Deoxynucleoside Triphosphate Set, Li-salt sol.	4 × 100 µl 4 × 100 µl	11 277 049 001 11 922 505 001
Single nucleotides	dATP, PCR Grade, Na-salt sol. Highest chemical purity	25 µmol, 250 µl 125 µmol, 1,250 µl 4 × 125 µmol, 4 × 1,250 µl	11 934 511 001 11 969 013 001 03 732 681 001
	dATP, Li-salt sol.	250 µl	11 051 440 001
	dCTP, PCR Grade, Na-salt sol. Highest chemical purity	25 µmol, 250 µl 125 µmol, 1,250 µl 4 × 125 µmol, 4 × 1,250 µl	11 934 520 001 11 969 021 001 03 732 690 001
	dCTP, Li-salt sol.	25 µmol, 250 µl	11 051 458 001
	dGTP PCR Grade, Na-salt sol. Highest chemical purity	25 µmol, 250 µl 125 µmol, 1,250 µl 4 × 125 µmol, 4 × 1,250 µl	11 934 538 001 11 969 030 001 03 732 703 001
	dGTP, Li-salt sol.	25 µmol, 250 µl	11 051 466 001
	dTTP PCR Grade, Na-salt sol. Highest chemical purity	25 µmol, 250 µl 125 µmol, 1,250 µl 4 × 125 µmol, 4 × 1,250 µl	11 934 546 001 11 969 048 001 03 732 711 001
	dTTP, Li-salt sol.	25 µmol, 250 µl	11 051 482 001
	dUTP PCR Grade, Na-salt sol. Highest chemical purity	25 µmol, 250 µl 125 µmol, 1,250 µl 4 × 125 µmol, 4 × 1,250 µl	11 934 554 001 11 969 056 001 03 732 720 001
	dUTP, Li-salt sol.	25 µmol, 250 µl	11 420 470 001
Ready-to-use mixes of all 4 nucleotides	PCR Nucleotide Mix PCR Grade, Na-salt sol.	200 µl 2,000 µl	11 581 295 001 11 814 362 001
Ready-to-use mixes of all 4 nucleotides, buffer and polymerases	Pwo Master	10 × 250 µl	03 789 403 001
	High Fidelity PCR Master	10 × 500 µl	12 140 314 001
	PCR Master	10 × 500 µl	11 636 103 001
	DOP PCR Master	3 × 500 µl	11 644 963 001

	Product	Pack Size	Cat. No.
Kits containing nucleotides, buffers and polymerases	PCR Core Kit	1 kit	11 578 553 001
	PCR Core Kit Plus	1 kit	11 585 541 001
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
Additional reagents	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
	PCR Cloning Kit (blunt end)	35 cloning and 5 control reactions	11 939 645 001
	0.2 ml thin-walled PCR tubes	1,000 tubes (200 µl) 1,000 tubes (500 µl)	11 667 041 001 11 667 050 001
	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

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