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

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 2500 U (10 × 250 U)

Version Dec. 2005
Store the kit 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 (2500 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 (2500 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 (2500 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 (2500 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.

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

* *available from Roche Applied Science*

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 unique 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 3-fold increase compared to Taq DNA polymerase.

Enzyme Properties

Volume activity	3.5 U/µl
Error rate[1]	3-fold 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 sterile double-distilled 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 µg/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 ml $MgCl_2$	5 µl	1× (1.5 ml $MgCl_2$)
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.