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

Expand Long Template PCR System

Version: 26
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Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase

Cat. No. 11 681 834 001	150 U 1 x 150 U 38 reactions in a final volume of 50 µl
Cat. No. 11 681 842 001	720 U 2 x 360 U 190 reactions in a final volume of 50 µl
Cat. No. 11 759 060 001	3,600 U 10 x 360 U 950 reactions in a final volume of 50 µl

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Expand Long Template PCR System, Enzyme Mix	Enzyme storage buffer: 20 mM Tris-HCl, pH 7.5 (+25°C), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% Nonidet P-40 (v/v), 0.5% Tween 20 (v/v), 50% glycerol (v/v).	11 681 834 001	1 vial, 30 µl
			11 681 842 001	2 vials, 72 µl each
			11 759 060 001	10 vials, 72 µl each
2	Expand Long Template PCR System, Buffer 1, 10x conc.	PCR buffer with 17.5 mM $MgCl_2$.	11 681 834 001	1 vial, 1 ml
			11 681 842 001	2 vials, 1 ml each
			11 759 060 001	10 vials, 1 ml each
3	Expand Long Template PCR System, Buffer 2, 10x conc.	PCR buffer with 27.5 mM $MgCl_2$.	11 681 834 001	1 vial, 1 ml
			11 681 842 001	2 vials, 1 ml each
			11 759 060 001	10 vials, 1 ml each
4	Expand Long Template PCR System, Buffer 3, 10x conc.	PCR buffer with 27.5 mM $MgCl_2$ and detergents.	11 681 834 001	1 vial, 1 ml
			11 681 842 001	2 vials, 1 ml each
			11 759 060 001	10 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Enzyme Mix	Once opened, store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	Buffer 1, 10x conc.	Store at −15 to −25°C. ⚠ ***Thaw and equilibrate at +37 to +56°C before use; vortex thoroughly. If crystals have formed, incubate at +37 to +56°C until dissolved.***
3	Buffer 2, 10x conc.	
4	Buffer 3, 10x conc.	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as 0.2 ml thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler

For PCR

- PCR primers
- Template DNA
- PCR Nucleotide Mix, PCR Grade*
- Water, PCR Grade*

1.4. Application

PCR and DNA labeling reactions

- Expand Long Template PCR System is optimized to efficiently amplify large genomic DNA fragments approximately 20 kb long.
- The 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 from genomic DNA.

> *Due to the inherent 3′→5′ exonuclease or proofreading activity of Tgo DNA polymerase, the fidelity of DNA synthesis with the Expand Long Template PCR System shows a threefold increase compared to Taq DNA polymerase.*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as 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 a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).
- Use up to 500 ng human genomic DNA.
  The quality of the template has a large effect on the success of the PCR.

Store the template DNA in either Water, PCR Grade* or 5 to 10 mM Tris-HCl, pH 7 to 8. Avoid dissolving the template in TE buffer since EDTA chelates Mg^{2+}.

Mg2+ Concentration

- 1.75 mM (as $MgCl_2$) when used with 350 µM of each dNTP (standard)
- 2.75 mM (as $MgCl_2$) when using 500 µM of each dNTP (standard)

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg^{2+} vary from system to system and must be determined for each individual experimental system. At the very least, titrate the Mg^{2+} concentration and the amount of enzyme mix used per assay to ensure optimal efficiency of DNA synthesis. If you are developing a new assay, you should test all three amplification systems to identify which gives the best results, see section, **Protocols**.

As a starting point, use the following guidelines:
- Optimal enzyme concentration: 0.5 to 5 U/50 µl. A concentration of 3.75 U (0.75 µl) will usually produce satisfactory results.
- Optimal Mg^{2+} concentration depends on the concentration of each dNTP. See section, **Mg^{2+} Concentration**.
- dNTP concentration: Always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 350 and 500 µM. If you increase the dNTP concentration, you must also increase the Mg^{2+} concentration.
- Use 0.2 ml thin-walled PCR tubes.
- Keep denaturation steps as short as possible and denaturation temperatures as low as possible.

Additives

Usually it is not necessary to add additives. 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 to 2% DMSO.

Prevention of Carryover Contamination

No
Unlabeled dUTP (instead of dTTP) is a poor substrate for the Expand enzyme mix. Therefore, do not use this product in combination with uracil-DNA glycosylase for carryover prevention.

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

2.2. Protocols

Preparation of PCR master mix

⚠ ***Always thaw and equilibrate all buffers at +37 to +56°C before use; vortex thoroughly.***

1 Briefly vortex and centrifuge all reagents before setting up the reactions.

2 To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:
– If the initial reaction produces too many primer-dimers, prepare two separate master mixes. Master Mix 1 contains dNTPs, primers, and template; Master Mix 2 contains buffer and enzyme. The final volume of each mix is 25 µl. For the final concentrations of each component, see the following table. Add these two Master Mixes to the tube on ice, then just before starting the reaction, vortex the tubes to produce a homogeneous reaction mix.

⚠ ***If the amplification system 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 genomic DNA	**0.5 – 9 kb Amplification System 1**		**9 – 12 kb Amplification System 2**		**>12 kb Amplification System 3**	
Reagent	**Volume [µl]**	**Final conc.**	**Volume [µl]**	**Final conc.**	**Volume [µl]**	**Final conc.**
Water, PCR Grade*	add up to a final volume of 50	–	add up to a final volume of 50	–	add up to a final volume of 50	–
dATP* (10 mM) (1)	1.75	350 µM	2.5	500 µM	2.5	500 µM
dCTP* (10 mM) (1)	1.75	350 µM	2.5	500 µM	2.5	500 µM
dGTP* (10 mM) (1)	1.75	350 µM	2.5	500 µM	2.5	500 µM
dTTP* (10 mM)(1)	1.75	350 µM	2.5	500 µM	2.5	500 µM
Forward primer 1	X	300 nM	X	300 nM	X	300 nM
Reverse primer 2	Y	300 nM	Y	300 nM	Y	300 nM
10x PCR Buffer with $MgCl_2$ (Buffers 1, 2, or 3)	5 (Buffer 1)	1.75 mM $MgCl_2$	5 (Buffer 2)	2.75 mM $MgCl_2$	5 (Buffer 3)	2.75 mM $MgCl_2$
Template DNA	Z	up to 500 ng genomic DNA	Z	up to 500 ng genomic DNA	Z	up to 500 ng genomic DNA
Enzyme Mix	0.75	–	0.75	–	0.75	–
Final Volume	**50**					

3 After pipetting the last reaction component, start the reactions immediately. Do not store on ice.

(1) Instead of using single dNTP solutions, use the PCR Grade Nucleotide Mix*.

PCR protocol

The following thermal profiles are an example. Different thermal cyclers may require different profiles. 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 profiles.

1. Place your samples in a thermal block cycler and start cycling using the following thermal profile.
   – The thermal profile has a gradually increasing extension time, ensuring a higher yield of amplification products.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92 – 94	2 min	1
Denaturation Annealing Elongation	92 – 94 45 – 65[1] 68	10 sec 30 sec 45 sec – 30 min[2]	10
Denaturation Annealing Elongation	92 – 94 45 – 65[1] 68	15 sec 30 sec 45 sec – 30 min[2] + 20 sec cycle elongation for each successive cycle[3]	15 – 25
Final Elongation	68	7 min	1
Cooling	4	indefinitely	

[1] Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.
[2] Elongation time depends on fragment length: 2 minutes for up to 3 kb, 4 minutes for 6 kb, 8 minutes for 10 kb, 15 minutes for 20 kb, and 20 minutes for 30 kb.
[3] For example, cycle number 11 is 20 seconds longer than cycle 10. Cycle number 12 is 40 seconds longer than cycle 10. Cycle number 13 is 60 seconds longer than cycle 10, etc.

2.3. Parameters

EC-Number

EC 2.7.7.7

Error Rate

Threefold more accurate compared to Taq DNA polymerase.
Relative fidelity determined by the lacI assay.

Incorporation of Modified Nucleotides

Enzyme blend accepts modified nucleotides, such as Digoxigenin-11-dUTP*, Biotin-16-dUTP*, or Fluorescein-12-dUTP*.

Maximum Fragment Size

Up to approximately 20 kb.

Proofreading Activity

Due to the 3′→5′ exonuclease activity of the proofreading polymerase, there is a repair of mismatched primers at the 3′ end.

Temperature Optimum

+68°C (elongation)

Volume Activity

5 U/µl

Working Concentration

0.5 to 5 U per 50 µl reaction (optimal).
3.75 U per 50 µl reaction (standard).
Optimal enzyme concentration varies from 0.5 to 5.0 U per 50 µl reaction. The standard enzyme concentration is 3.75 U per 50 µl reaction.

3. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	Difficult templates, such as GC-rich templates.	Try Amplification System 2 or 3 first, even if you have short amplicons. *See section,* **Protocols, Preparation of PCR master mix**.
	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 template purification.
	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; the minimal acceptable concentration is 1.75 mM $MgCl_2$.
	Cycle conditions not optimal.	Decrease annealing temperature.
		Increase cycle number.
		Make sure that the final elongation step is included in the program.
	Primer design not optimal.	Design alternative primers.
	Primer concentration not optimal.	Both primers must have the same concentration.
		Titrate primer concentration (0.3 to 0.6 µM).
	Annealing temperature too high.	Reduce annealing temperature (minimum annealing temperature is +45°C).
		Determine the optimal annealing temperature by touchdown PCR.
	Primer quality or storage problems.	If you use an established primer pair, check performance in an established PCR system, for example, with a control template.
		Make sure that 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 primer length.
	Primer design or concentration not optimal.	Review primer design.
		Titrate primer concentration (0.3 to 0.6 µM).
		Both primers must have the same concentration.
		Perform nested PCR with nested primers.
		Primers should have similar melting temperatures.
	DNA template problems.	Use serial dilution of template.
PCR products in negative control experiments.	Carryover contamination present.	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.
Problems specific to RT-PCR.	No product, additional bands, background smear observed.	The volume of cDNA template (RT reaction) should not exceed 10% of the final concentration of the PCR reaction.
		Follow all troubleshooting tips.
		Increase $MgCl_2$ in 0.25 mM steps.

4. Additional Information on this Product

4.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Biotin-16-dUTP	custom fill	11 093 711 103
Fluorescein-12-dUTP	custom fill	11 375 601 103
Digoxigenin-11-dUTP, alkali-stable	25 nmol, 25 µl, 1 mM	11 093 088 910
	125 nmol, 125 µl, 1 mM	11 558 706 910
	5 x 125 nmol, 5x 125 µl, 1 mM	11 570 013 910
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910

5.4. Trademarks

MAGNA PURE and EXPAND are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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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