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

Expand Long Range dNTPack

Version: 09
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Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase

Cat. No. 04 829 034 001	175 U 1 x 175 U 50 reactions in a final volume of 50 µl
Cat. No. 04 829 042 001	700 U 1 x 700 U 200 reactions in a final volume of 50 µl
Cat. No. 04 829 069 001	3,500 U 5 x 700 U 1,000 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 Range dNTPack, Polymerase Blend	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).	04 829 034 001	1 vial, 35 µl
			04 829 042 001	1 vial, 140 µl
			04 829 069 001	5 vials, 140 µl each
2	Expand Long Range dNTPack, PCR Buffer with $MgCl_2$, 5x conc.	PCR Buffer with 12.5 mM $MgCl_2$.	04 829 034 001	1 vial, 1 ml
			04 829 042 001	2 vials, 1 ml each
			04 829 069 001	10 vials, 1 ml each
3	Expand Long Range dNTPack, PCR Buffer without $MgCl_2$, 5x conc.	PCR Buffer without $MgCl_2$.	04 829 034 001	1 vial, 1 ml
			04 829 042 001	2 vials, 1 ml each
			04 829 069 001	10 vials, 1 ml each
4	Expand Long Range dNTPack, $MgCl_2$ 25 mM Stock Solution	To adjust final Mg^{2+} concentration.	04 829 034 001	1 vial, 1 ml
			04 829 042 001	1 vial, 1 ml
			04 829 069 001	5 vials, 1 ml each
5	Expand Long Range dNTPack, DMSO	100% DMSO	04 829 034 001	1 vial, 1 ml
			04 829 042 001	1 vial, 1 ml
			04 829 069 001	5 vials, 1 ml each
6	Expand Long Range dNTPack, PCR Nucleotide Mix	Contains dATP, dCTP, dGTP, and dTTP at 10 mM each.	04 829 034 001	1 vial, 200 µl
			04 829 042 001	3 vials, 200 µl each
			04 829 069 001	13 vials, 200 µl 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	Polymerase Blend	Store at −15 to −25°C.
2	PCR Buffer with $MgCl_2$, 5x conc.	
3	PCR Buffer without $MgCl_2$, 5x conc.	
4	$MgCl_2$ 25 mM Stock Solution	
5	DMSO	
6	PCR Nucleotide Mix	

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
- Water, PCR Grade*
- Mineral oil or self-adhesive foil (optional)

1.4. Application

PCR and DNA labeling reactions

Expand Long Range dNTPack is optimized to efficiently amplify large genomic DNA fragments from 5 to 25 kb (or up to 40 kb for λ DNA templates) in combination with a threefold higher fidelity than Taq DNA polymerase.
The kit is ideally suited for:

- Genome mapping and sequencing.
- Contig construction
- Characterization of cloned sequences in lambda phages or cosmids.
- Eukaryotic gene cloning and analysis.
- Rapid identification and cloning of whole genes from genomic DNA using cDNA sequence information as a starting point.

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 complex genomic DNA or 1 pg to 100 ng plasmid DNA, cDNA, or bacterial DNA.

  In initial experiments to determine the optimum amount of cDNA template, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel. Adding too much cDNA may inhibit the PCR.

Store the template DNA in either sterile double-distilled water, 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+}.

Control Reactions

Negative control

Take into consideration that even with long PCR products, cross-contamination can occur.
To detect DNA contamination, always include a negative control in each run. To prepare this control, replace template DNA with Water, PCR Grade*. Sometimes a contamination will not lead to a prominent band, but will be visible as a smear in the lane of the agarose gel.

Primers

Primer design is crucial for the performance of long-range PCR. Follow these general guidelines:
- Primers should be between 22 to 34 nucleotides long.
- GC-content should be in the range of 45 to 65%.
- Optimally, there should be 2 G's or C's at the 3′ end of the primer.
- Avoid high similarity (>80%) to other sequences in the genome.
- Check primers for possible primer-dimer formation.

If there are difficulties to amplify the sequence of interest, sometimes designing different primers or using different primer pairs helps to overcome the problem.

Mg2+ Concentration

2.5 mM final Mg^{2+} concentration

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg^{2+}, and DMSO concentration vary from system to system and must be determined for each individual experimental system.
As a starting point, use the following guidelines:
- Optimal enzyme concentration: 2.5 to 5 U/50 µl. A concentration of 3.5 U will usually produce satisfactory results.

  For specific applications, it may be necessary to increase the enzyme concentration up to 5 U per assay, for example, for multiplex PCR.
- For every new assay, determine the optimal DMSO concentration. Depending on the GC content, the secondary structure, and other amplicon-specific parameters, the addition of DMSO improves the yield in long range PCR. Start with a concentration of 3% DMSO. The optimal concentration may vary from 0 to 12%.
- Use 0.2 ml thin-walled PCR tubes.

Additives

Usually it is not necessary to add additives. In some cases, improvements can be achieved by using additives, such as 7-deaza-dGTP, PEG 1,000 or 6,000, Tween 20 (v/v), glycerol, or T4 Gene 32 Protein to improve the PCR performance.

Templates with high GC content

Keep denaturation steps as short as possible and denaturation temperature as low as possible. Sometimes fragments with high GC content need higher denaturation temperatures. The enzyme mix is compatible with denaturation temperatures up to +96°C; however, the yield increases when denaturation temperature is decreased.

Templates with low GC content

For templates with a very low GC content, reducing the elongation temperature can sometimes help to get an amplification product.

Template quality

The quality of the template has a tremendous effect on the success of the PCR. Prepare template DNA carefully, applying gentle methods that do not shear the template in order to yield high molecular weight DNA of high purity.
⚠ ***Never vortex complex genomic DNA. Always store complex genomic DNA at +2 to +8°C. Avoid repeated freeze-thaw steps.***

Reaction volume

Use Expand Long Range dNTPack for a variety of reaction volumes. Refer to the supplier's recommendations of the thermal block cycler for suitable volumes, tube size, and plate formats.

Prevention of Carryover Contamination

No
The kit is not compatible with carryover prevention using the dUTP/uracil-DNA glycosylase procedure.

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

1. Always thaw and equilibrate the required buffers and DMSO at +37°C for 10 minutes before use.
   – If precipitates have formed during storage, incubate at +37°C until they are completely dissolved; vortex thoroughly.
   Place all components on ice, except the DMSO; keep DMSO at +15 to +25°C.

2. For maximum recovery of the contents, briefly spin vials in a microcentrifuge before opening.

3. Mix template DNA carefully to avoid shearing the DNA; do not vortex.

4. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:
   To prepare the PCR mix for more than one reaction, multiply the amounts in the Volume column by z, where z equals the number of reactions to be run plus one additional reaction.

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to a final volume of 50	–
PCR Buffer with $MgCl_2$, 5x conc. (Vial 2)	10	1x
PCR Nucleotide Mix (10 mM of each dNTP) (Vial 6)	2.5	500 µM of each dNTP
Forward primer 1	variable	300 nM
Reverse primer 2	variable	300 nM
DMSO	0 – 6	0 – 12%
Polymerase Blend (Vial 1)	0.7	3.5 U/reaction
Template DNA	variable	up to 500 ng
Final Volume	**50**	

5. Mix solution carefully by pipetting up and down; do not vortex.

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

PCR protocol

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

1. Pipette 50 µl of the PCR mix into each PCR reaction vessel or well of a PCR multiwell plate, depending on the block cycler instrument.
   Overlay the reaction carefully with mineral oil or seal the plate with a self-adhesive foil if required by the thermal cycler.

2. Place your samples in a thermal block cycler and start cycling using the following thermal profile.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	92(1)	2 min	1
Denaturation Annealing Elongation	92(1) 45 – 65(2) 68	10 sec 15 sec 60 sec/kb(3)	10
Denaturation Annealing Elongation	92(1) 45 – 65(2) 68	10 sec 15 sec 60 sec/kb(3) + 20 sec cycle elongation for each successive cycle	15 – 25
Final Elongation	68	up to 7 min	1
Cooling	8	indefinitely	

(1) Optimal denaturation temperature depends on the GC content of the amplified fragment. Sometimes denaturation temperatures up to +96°C may be necessary to amplify a fragment with high secondary structure.
(2) Optimal annealing temperature depends on the melting temperature of the primers and the target sequence used.
(3) Elongation time depends on amplicon length. Start with an elongation time of 1 minute/kb.

2.3. Parameters

EC-Number

EC 2.7.7.7

Error Rate

Approximately threefold higher fidelity of DNA synthesis compared to Taq DNA polymerase.

Incorporation of Modified Nucleotides

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

Maximum Fragment Size

From 5 to 25 kb when using human genomic DNA as template.

PCR Cloning

Expand Long Range dNTPack-generated PCR products can be used both for TA cloning or blunt-end ligation.

Proofreading Activity

Yes

Temperature Optimum

+68°C (elongation)
+92°C (denaturation)

Volume Activity

5 U/µl

3. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	Difficult templates, such as GC-rich templates.	Titrate the DMSO.
		Depending on the GC content, it may be necessary to raise the denaturation/annealing temperature to improve the yield.
		For fragments with a very low GC content, reduction of the elongation temperature down to +58°C can help to obtain a product.
	Poor DNA template quality.	Check quality and concentration of template: ▪ Analyze an aliquot on an agarose gel (0.5%. 60 V, 5 hours) to check for possible degradation. ▪ Include a control reaction using a known template under established PCR conditions. ▪ Check or repeat template purification.
	$MgCl_2$ concentration too low.	Increase the $MgCl_2$ concentration in 0.25 mM steps from 1.75 mM up to 3.5 mM.
	Cycle conditions not optimal.	Decrease annealing temperature.
		Increase cycle number.
	Primer design not optimal.	Design alternative primers.
Multiple bands or background smear.	Annealing temperature too low.	Increase annealing temperature; maximum annealing temperature is +68°C.
		Determine the optimal annealing temperature by using a gradient.
	Contamination with old PCR product.	Replace all reagents.
		Set up PCR reactions in an area separate from that used for PCR product analysis, such as a flow box.
	Primer quality or storage problems.	If you are using an established primer pair, check their performance under established PCR conditions using a control template.
		Make sure primers are not degraded.
		Always store primer in aliquots at −15 to −25°C.
	Primer design or concentration not optimal.	Review primer design.
		Both primers must have the same concentration.
		Primers should have similar melting temperatures.
	DNA template problems.	Use serial dilution of template.
		Check quality and purity of the DNA.
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. Test Principle

Taq DNA polymerase, the most common PCR enzyme, is able to amplify up to 15 kb from λ DNA templates and up to 3 kb from complex genomic templates. Using a mixture of Taq DNA polymerase and a thermostable DNA polymerase with proofreading (3′→5′ exonuclease) activity could overcome limitations in the length of fragments amplified. The proofreading enzyme can remove misincorporated nucleotides from the 3′ end of the growing DNA strand. Such misincorporations would otherwise cause the Taq DNA polymerase to fall off the DNA strand and lead to the generation of truncated PCR products. The Expand PCR Systems combine the processivity of Taq DNA polymerase with the accuracy of a proofreading polymerase.

4.2. 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		
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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