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

Expand High Fidelity^{PLUS} PCR System, dNTPack

Version: 08
Content Version: November 2020

Expand High Fidelity enzyme blend with additional ready-to-use 10 mM PCR Grade Nucleotide Mix.

Cat. No. 04 743 725 001	125 U 1 x 125 U 50 reactions in a final volume of 50 µl
Cat. No. 04 743 733 001	500 U 2 x 250 U 200 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 High Fidelity^{PLUS} PCR System, dNTPack, Enzyme Blend	Enzyme storage buffer: 20 mM Tris-HCl, pH 8 (+4°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).	04 743 725 001	1 vial, 25 µl
			04 743 733 001	2 vials, 50 µl each
2	Expand High Fidelity^{PLUS} PCR System, dNTPack, Reaction Buffer, 5x conc. with $MgCl_2$	Reaction buffer with 7.5 mM $MgCl_2$.	04 743 725 001	1 vial, 1.25 ml
			04 743 733 001	2 vials, 1.25 ml each
3	Expand High Fidelity^{PLUS} PCR System, dNTPack, Reaction Buffer, 5x conc. without $MgCl_2$	Reaction buffer without $MgCl_2$.	04 743 725 001	1 vial, 1.25 ml
			04 743 733 001	2 vials, 1.25 ml each
4	Expand High Fidelity^{PLUS} PCR System, dNTPack, $MgCl_2$ 25 mM Stock Solution	To adjust final Mg^{2+} concentration.	04 743 725 001	1 vial, 1 ml
			04 743 733 001	2 vials, 1 ml each
5	Expand High Fidelity^{PLUS} PCR System, dNTPack, PCR Grade Nucleotide Mix	Ready-to-use 10 mM dNTP solution.	04 743 725 001	1 vial, 200 µl
			04 743 733 001	1 vial, 200 µl

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 Blend	Store at −15 to −25°C.
2	Reaction Buffer, 5x conc. with $MgCl_2$	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	Reaction Buffer, 5x conc. without $MgCl_2$	
4	$MgCl_2$ 25 mM Stock Solution	Store at −15 to −25°C.
5	PCR Grade 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*

For incorporation of nonradioactive modified nucleotides

- Biotin-16-dUTP*
- Fluorescein-12-dUTP*
- Digoxigenin-11-dUTP, alkali-stable*
- Digoxigenin-11-dUTP, alkali-labile*
- Deoxynucleoside Triphosphate Set, PCR Grade* (optional)

For carryover prevention (optional)

- Uracil-DNA Glycosylase, heat-labile*
- PCR Nucleotide Mix^{PLUS}, PCR Grade*

1.4. Application

The Expand High Fidelity^{PLUS} PCR System, dNTPack is designed to:

- Amplify fragments up to 5 kb from all types of DNA with outstanding yield and fidelity.
  - *The system is a blend of Taq DNA Polymerase and a thermostable proofreading protein that lacks polymerase activity.*
- Amplify DNA with twofold greater replicational accuracy (fidelity) than the Expand High Fidelity PCR System, and sixfold greater fidelity than Taq DNA Polymerase alone.
  - *The synergy between this proofreading protein and the highly processive Taq DNA Polymerase is the key to the outstanding yield, specificity, sensitivity, and accuracy of the Expand High Fidelity^{PLUS} System.*
- Incorporate dUTP and, in combination with Uracil-DNA Glycosylase, can be used to safeguard PCR reactions from cross-contamination. Therefore, it is suited for simultaneous amplification of a large number of different targets in the same run.

These characteristics make the Expand High Fidelity^{PLUS} PCR System blend the product of choice if a larger number of targets need to be amplified at the same time with high yield in combination with improved accuracy.

- Efficiently label DNA fragments with radioactive or nonradioactive modified nucleotides.

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 5 to 500 ng human genomic DNA or 0.1 to 10 ng plasmid DNA/cDNA. Use a starting concentration of 250 ng genomic DNA or 1 ng plasmid DNA.

⚠ ***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+}.***

Primers

Forward and reverse primer
- Start with 0.4 µM (final concentration) of each primer.
- For optimization, the concentration can vary between 0.2 and 0.6 µM.

Mg2+ Concentration

1.5 to 4 mM (as $MgCl_2$) (optimal)
1.5 mM (as $MgCl_2$) when used with 200 µM of each dNTP (standard)

ⓘ *The Mg^{2+} concentration must be optimized when dUTP is used.*

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. As a starting point, use the following guidelines:
- Optimal enzyme concentration: 2.5 U/50 µl.
- Optimal Mg^{2+} concentration can vary between 1.5 to 4 mM. In most cases, a Mg^{2+} concentration of 1.5 mM will produce satisfactory results if you use 200 µM of each dNTP.

Prevention of Carryover Contamination

Yes

PCR with carryover prevention (optional)

The magnesium concentration must be optimized for each individual target:
- Use Expand High Fidelity^{PLUS} Reaction Buffer, 5x conc. without $MgCl_2$ (Vial 3) and $MgCl_2$ 25 mM Stock Solution (Vial 4) in concentrations ranging from 1.5 mM to 4 mM final concentration.
- Add Uracil-DNA Glycosylase, heat labile*: 2 units per reaction (2 µl).
- Use PCR Nucleotide Mix^{PLUS}*: 200 µM dATP, dCTP, dGTP, and 600 µM dUTP final concentration.

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

See optional sections, **Prevention of Carryover Contamination** *and* **Incorporation of Modified Nucleotides** *for additional information.*

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

2. Prepare a 10x-concentrated solution of each respective primer.
   If you are using, for example, the final concentration of 0.4 µM for each primer, the 10x-concentrated solution would contain a 4 µM concentration of the respective primer.

3. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade* or double-distilled water	add up to a final volume of 50	–
Reaction Buffer, 5x conc. with $MgCl_2$ (Vial 2)[1][2]	10	1.5 mM $MgCl_2$[1][2]
PCR Grade Nucleotide Mix (10 mM of each dNTP)	1[1][2]	200 µM of each dNTP[1][2]
Forward primer 1, 10x conc.	5	0.4 µM
Reverse primer 2, 10x conc.	5	0.4 µM
Template DNA	variable	5 – 500 ng genomic DNA, 100 pg – 10 ng plasmid DNA
Enzyme Blend (Vial 1)	0.5	2.5 U/reaction
Final Volume	**50**	

4. Gently vortex the mixture to produce a homogeneous reaction.

5. For decontamination[1] using dUTP/Uracil-DNA Glycosylase, heat-labile (optional), preincubate the reaction for 10 minutes at +20°C, then proceed to thermal cycling.

[1] See section, **Prevention of Carryover Contamination**.
[2] See section, **Incorporation of Modified Nucleotides**.

PCR protocol

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

1. Place your samples in a thermal block cycler and use either of the thermal profiles below to perform PCR.

Thermal Profile A has a fixed extension time.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94[1]	2 min	1
Denaturation Annealing Elongation	94[1] 55 – 68[2] 68 – 72[3]	10 – 30 sec 30 sec 30 sec – 4 min[4]	25 – 35
Final Elongation	68 – 72[3]	7 min	1

Thermal Profile B 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	94[1] 55 – 68[2] 68 – 72[3]	10 – 30 sec 30 sec 30 sec – 4 min[4]	10
Denaturation Annealing Elongation	94[1] 55 – 68[2] 68 – 72[3]	10 – 30 sec 30 sec 30 sec – 4 min[4] + 10 sec cycle elongation for each successive cycle[5]	15 – 25
Final Elongation	68 – 72[3]	7 min	1
Cooling	4	indefinitely	

2. After cycling, use samples immediately or store them frozen for later use.

For best results, check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker.

(1) Optimal denaturation temperature and time depends upon the GC content of the template.
(2) Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.
(3) For PCR products up to 3 kb, elongation temperature should be approximately +72°C; for PCR products >3 kb, elongation temperature should be approximately +68°C.
(4) Elongation time depends upon the length of the product to be amplified, approximately 1 minute per kb.
(5) For example, cycle number 11 is 10 seconds longer than cycle 10. Cycle number 12 is 20 seconds longer than cycle 10. Cycle number 13 is 30 seconds longer than cycle 10, etc.

2.3. Parameters

Error Rate

- Twofold more accurate compared to the Expand High Fidelity PCR System.
- Sixfold more accurate compared to Taq DNA polymerase.

Relative fidelity determined by the lacI assay.

Incorporation of Modified Nucleotides

Accepts modified nucleotides, such as Digoxigenin-dUTP, Biotin-dUTP, and Fluorescein-dUTP.
To use the Expand High Fidelity^{PLUS} PCR system to incorporate nonradioactive modified nucleotides, exchange the PCR Nucleotide Mix with one of the following modified nucleotide mixes:

Nucleotide Mix, 10x conc.	Description/Preparation
Digoxigenin-11-dUTP	▪ 0.7 mM Digoxigenin-11-dUTP*, 1.3 mM dTTP, 2 mM dCTP, 2 mM dATP, and 2 mM dGTP. ▪ Use a final $MgCl_2$ concentration of 3 mM, however it is best to optimize the concentration for each new reaction.
Biotin-16-dUTP	▪ 0.7 mM Biotin-16-dUTP*, 1.3 mM dTTP, 2 mM dCTP, 2 mM dATP, and 2 mM dGTP. ▪ Use a final $MgCl_2$ concentration of 1.5 mM.
Fluorescein-12-dUTP	▪ 0.5 mM Fluorescein-12-dUTP*, 1.5 mM dTTP, 2 mM dCTP, 2 mM dATP, and 2 mM dGTP. ▪ Use a final $MgCl_2$ concentration of 1.5 mM.

For convenience, use the Deoxynucleoside Triphosphate Set, PCR Grade which contains individual vials of dATP, dCTP, dGTP, dTTP at a concentration of 100 mM.*

Maximum Fragment Size

Up to 5 kb.

PCR Cloning

TA cloning
Enzyme adds a single, overhanging adenine (A).

Proofreading Activity

Yes

Temperature Optimum

+72°C (elongation) for amplicons up to 3 kb.
For PCR products >3 kb, the optimal elongation temperature is +68°C.

Volume Activity

5 U/µl

Working Concentration

2.5 U per 50 µl reaction (standard).

3. Troubleshooting

Observation	Possible cause	Recommendation
Little or no PCR product.	Pipetting errors	Check all concentrations and storage conditions of reagents.
	Difficult templates, such as GC-rich templates.	Add DMSO (final concentration 8%). *DMSO may negatively influence the accuracy of the reaction.*
		Perform PCR with 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. ▪ Perform a control reaction on template using an established primer pair. ▪ 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. *Minimum concentration is 1.5 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 (0.2 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 specificity not optimal.	Perform nested PCR.
	Primer quality or storage problems.	If you use an established primer pair, check performance 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 (0.2 to 0.6 µM).
		Both primers must have the same concentration.
		Perform nested PCR with nested primers.
	Difficult templates, such as GC-rich templates.	Perform PCR with the GC-RICH PCR System*.
	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.
		To eliminate carryover contaminants: ▪ Use dUTP (600 µM) instead of dTTP (200 µM), and Uracil-DNA Glycosylase* (2 U/50 µl reaction). ▪ Increase Mg^{2+} concentration to a maximum of 4 mM.

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^{PLUS}	2 x 100 µl, 200 PCR reactions in 50 µl	11 888 412 001
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
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
GC-RICH PCR System	100 U, 50 reactions in a final volume of 50 µl	12 140 306 001
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
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001

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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany