

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



# Expand High Fidelity PCR System, dNTPack

 **Version: 08**

Content Version: October 2020

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

<b>Cat. No. 04 738 250 001</b>	100 U 1 x 100 U 40 reactions in a final volume of 50 µl
<b>Cat. No. 04 738 268 001</b>	500 U 2 x 250 U 200 reactions in a final volume of 50 µl
<b>Cat. No. 04 738 276 001</b>	2,500 U 10 x 250 U 1,000 reactions in a final volume of 50 µl

**Store the kit at –15 to –25°C.**

<b>1.</b>	<b>General Information .....</b>	<b>3</b>
1.1.	Contents .....	3
1.2.	Storage and Stability .....	4
	Storage Conditions (Product) .....	4
1.3.	Additional Equipment and Reagent required .....	4
1.4.	Application .....	4
	PCR and DNA labeling reactions .....	4
<b>2.</b>	<b>How to Use this Product .....</b>	<b>5</b>
2.1.	Before you Begin .....	5
	Sample Materials .....	5
	Mg <sup>2+</sup> Concentration .....	5
	General Considerations .....	5
	Additives .....	5
	Prevention of Carryover Contamination .....	5
	Safety Information .....	5
	For customers in the European Economic Area .....	5
2.2.	Protocols .....	6
	Preparation of PCR master mixes .....	6
	PCR protocol .....	7
2.3.	Parameters .....	8
	Error Rate .....	8
	Incorporation of Modified Nucleotides .....	8
	Maximum Fragment Size .....	8
	PCR Cloning .....	8
	Proofreading Activity .....	8
	Temperature Optimum .....	8
	Volume Activity .....	8
	Working Concentration .....	8
<b>3.</b>	<b>Troubleshooting .....</b>	<b>9</b>
<b>4.</b>	<b>Additional Information on this Product .....</b>	<b>10</b>
4.1.	Quality Control .....	10
<b>5.</b>	<b>Supplementary Information .....</b>	<b>10</b>
5.1.	Conventions .....	10
5.2.	Changes to previous version .....	10
5.3.	Ordering Information .....	10
5.4.	Trademarks .....	11
5.5.	License Disclaimer .....	11
5.6.	Regulatory Disclaimer .....	11
5.7.	Safety Data Sheet .....	11
5.8.	Contact and Support .....	11

# 1. General Information

## 1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Expand High Fidelity PCR System, dNTPack, 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).	04 738 250 001	1 vial, 30 µl
			04 738 268 001	2 vials, 75 µl each
			04 738 276 001	10 vials, 75 µl each
2	Expand High Fidelity PCR System, dNTPack, PCR buffer, 10x conc. with MgCl <sub>2</sub>	PCR buffer with 15 mM MgCl <sub>2</sub> .	04 738 250 001	1 vial, 1 ml
			04 738 268 001	2 vials, 1 ml each
			04 738 276 001	10 vials, 1 ml each
3	Expand High Fidelity PCR System, dNTPack, PCR buffer, 10x conc. without MgCl <sub>2</sub>	PCR buffer without MgCl <sub>2</sub> .	04 738 250 001	1 vial, 1 ml
			04 738 268 001	1 vial, 1 ml
			04 738 276 001	10 vials, 1 ml each
4	Expand High Fidelity PCR System, dNTPack, MgCl <sub>2</sub> 25 mM Stock Solution	To adjust final Mg <sup>2+</sup> concentration.	04 738 250 001	1 vial, 1 ml
			04 738 268 001	1 vial, 1 ml
			04 738 276 001	10 vials, 1 ml each
5	Expand High Fidelity PCR System, dNTPack, PCR Grade Nucleotide Mix	Ready-to-use 10 mM dNTP solution.	04 738 250 001	1 vial, 200 µl
			04 738 268 001	1 vial, 200 µl
			04 738 276 001	5 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	Enzyme Mix	Store at –15 to –25°C.
2	PCR buffer, 10x conc. with MgCl <sub>2</sub>	Store at –15 to –25°C.
3	PCR buffer, 10x conc. without MgCl <sub>2</sub>	<b>⚠ Thaw and equilibrate at +37 to +56°C before use; vortex thoroughly. If crystals have formed, incubate at +37 to +56°C until dissolved.</b>
4	MgCl <sub>2</sub> 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\*

### 1.4. Application

#### 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.

**i** For the generation of longer PCR products, use the Expand Long Template PCR System\*, which is optimized for the amplification of DNA fragments ranging from 3 kb to 27 kb in length.

- Expand High Fidelity PCR 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, high fidelity, and high specificity from all types of DNA.

**i** Due to the inherent 3'→5' exonuclease or proofreading activity of Tgo DNA polymerase, the fidelity of DNA synthesis with the Expand High Fidelity 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 10 to 250 ng human genomic DNA or 0.1 to 15 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<sup>2+</sup>.**

#### Mg<sup>2+</sup> Concentration

1.5 to 4 mM (as MgCl<sub>2</sub>) (optimal)

1.5 mM (as MgCl<sub>2</sub>) when used with 200 μM of each dNTP (standard)

#### General Considerations

The optimal conditions, including incubation times and temperatures, concentration of enzyme, template DNA, Mg<sup>2+</sup> vary from system to system and must be determined for each individual experimental system. At the very least, titrate the Mg<sup>2+</sup> 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: 0.5 to 5 U/50 μl. A concentration of 2.6 U (0.75 μl) will usually produce satisfactory results.
- Optimal Mg<sup>2+</sup> concentration can vary between 1.5 to 4 mM. In most cases, a Mg<sup>2+</sup> concentration of 1.5 mM will produce satisfactory results if you use 200 μM of each dNTP.

#### 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 mixes

Prepare two PCR master mixes. Master Mix 2 contains enzyme and reaction buffer; Master Mix 1 contains all other reaction components. This circumvents the need for hot start and avoids that the enzyme interacts with primers or template through the 3'→5' exonuclease activity of Tgo DNA Polymerase. If you are setting up multiple reactions, the volume of each master mix should be 110% of the volume needed for all the samples. For example, to prepare Master Mix 2 for 10 reactions, make 275 µl of the mix. The extra volume allows for losses during pipetting.

#### Preparation of master mix 1

- 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.
    - i* If you are using, for example, the final concentration of 0.3 µM for each primer, the 10x-concentrated solution would contain a 3 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*	add up to a final volume of 25	–
PCR Grade Nucleotide Mix (10 mM of each dNTP)	1	200 µM of each dNTP
Forward primer 1	5	300 nM
Reverse primer 2	5	300 nM
Template DNA	variable	0.1 – 250 ng
<b>Final Volume</b>	<b>25</b>	

- 4 Mix and centrifuge briefly.
- 

#### Preparation of master mix 2

- 1 Thaw the reagents and store on ice.
    - 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:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	19.25	–
PCR buffer, 10x conc. with MgCl <sub>2</sub> (Vial 2)	5	1x (1.5 mM MgCl <sub>2</sub> )
Enzyme Mix (Vial 1)	0.75	2.6 U/reaction
<b>Final Volume</b>	<b>25</b>	

*i* When titrating the Mg<sup>2+</sup> concentration, use the PCR buffer, 10x conc. without MgCl<sub>2</sub> (Vial 3) and the MgCl<sub>2</sub> 25 mM Stock Solution (Vial 4).

- 3 Mix and centrifuge briefly.
-

## PCR protocol

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

- 1** For each reaction, combine 25 µl Master Mix 1 and 25 µl Master Mix 2 in a thin-walled PCR tube on ice.
  - Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.
- 2** 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.

**i** The elongation step should be performed at +68°C when amplifying PCR products >3 kb.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	2 min	1
Denaturation	94	15 sec	10
Annealing	45 – 65 <sup>(1)</sup>	30 sec	
Elongation	72 <sup>(2)</sup> or 68	45 sec – 8 min <sup>(3)</sup>	
Denaturation	94	15 sec	15 – 20
Annealing	45 – 68 <sup>(1)</sup>	30 sec	
Elongation	72 <sup>(2)</sup>	45 sec – 8 min <sup>(3)</sup> + 5 sec cycle elongation for each successive cycle <sup>(4)</sup>	
Final Elongation	72	7 min	1
Cooling	4	indefinitely	

- 3** After cycling, use samples immediately or store them frozen for later use.

**i** For best results, check the PCR product on an agarose gel for size and specificity. Use an appropriate size marker. In addition, purify the PCR product with the High Pure PCR Product Purification Kit, for example, before performing nested PCR.

- <sup>(1)</sup> Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.
- <sup>(2)</sup> For PCR products up to 3 kb, elongation temperature should be +72°C; for PCR products >3 kb, elongation temperature should be +68°C.
- <sup>(3)</sup> Elongation time depends on fragment length: 45 seconds for up to 0.75 kb, 1 minute for 1.5 kb, 2 minutes for 3 kb, 4 minutes for 6 kb, and 8 minutes for 10 kb.
- <sup>(4)</sup> For example, cycle number 11 is 5 seconds longer than cycle 10. Cycle number 12 is 10 seconds longer than cycle 10. Cycle number 13 is 15 seconds longer than cycle 10, etc.

### 2.3. Parameters

#### Error Rate

Threefold more accurate compared to Taq DNA polymerase.

**i** *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\*.

- For generating probes for Southern analysis, use a concentration of 50  $\mu\text{M}$  modified dUTP (with 150  $\mu\text{M}$  dTTP).
- When using Fluorescein-dUTP, increase the  $\text{MgCl}_2$  concentration to 4 mM.
- For ELISA based detection systems, a concentration of 10  $\mu\text{M}$  modified dUTP is sufficient.

#### Maximum Fragment Size

Up to 5 kb.

#### PCR Cloning

TA cloning

Enzyme adds a single, overhanging adenine (A).

#### 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

+72°C (elongation)

For PCR products >3 kb, the optimal elongation temperature is +68°C.

#### Volume Activity

3.5 U/ $\mu\text{l}$

#### Working Concentration

0.5 to 5 U per 50  $\mu\text{l}$  reaction (optimal).

2.6 U (0.75  $\mu\text{l}$ ) per 50  $\mu\text{l}$  reaction (standard).



## 3. Troubleshooting

Observation	Possible cause	Recommendation	
Little or no PCR product.	Difficult templates, such as GC-rich templates.	Perform Pre-Incubation step at +95°C for 3 to 5 minutes.	
		Perform PCR with the GC-RICH PCR System*.	
	Poor DNA template quality.	Check quality and concentration of template: <ul style="list-style-type: none"> <li>▪ Analyze an aliquot on an agarose gel to check for possible degradation.</li> <li>▪ Include a control reaction using a known template under established PCR conditions.</li> <li>▪ Check or repeat template purification.</li> </ul>	
		Enzyme concentration too low.	Increase the amount of enzyme mix in 0.5 U steps.
		MgCl <sub>2</sub> concentration too low.	Increase the MgCl <sub>2</sub> concentration in 0.25 mM steps; the minimal acceptable concentration is 1.5 mM MgCl <sub>2</sub> .
	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 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.		
Formation of primer-dimers.	Use two master mixes, as described in the protocol.		
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.	
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 <sub>2</sub> 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.	
 <b>Important Note: Information critical to the success of the current procedure or use of the product.</b>	
   etc.	Stages in a process that usually occur in the order listed.
   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
GC-RICH PCR System	100 U, 50 reactions in a final volume of 50 µl	12 140 306 001
Expand Long Template PCR System	150 U, 1 x 150 U, 38 reactions in a final volume of 50 µl	11 681 834 001
	720 U, 2 x 360 U, 190 reactions in a final volume of 50 µl	11 681 842 001
	3,600 U, 10 x 360 U, 950 reactions in a final volume of 50 µl	11 759 060 001
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.

