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

# Expand High Fidelity PCR System

**Version: 22**
Content Version: November 2020

Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase

| | |
|---|---|
| **Cat. No. 11 732 641 001** | 100 U<br>1 x 100 U<br>40 reactions in a final volume of 50 µl |
| **Cat. No. 11 732 650 001** | 500 U<br>2 x 250 U<br>200 reactions in a final volume of 50 µl |
| **Cat. No. 11 759 078 001** | 2,500 U<br>10 x 250 U<br>1,000 reactions in a final volume of 50 µl |

**Store the kit at −15 to −25°C.**

| | | |
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# 1. General Information

## 1.1. Contents

| Vial / bottle | Label | Function / description | Catalog number | Content |
|---|---|---|---|---|
| 1 | Expand High Fidelity 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). | 11 732 641 001 | 1 vial, 30 µl |
| | | | 11 732 650 001 | 2 vials, 75 µl each |
| | | | 11 759 078 001 | 10 vials, 75 µl each |
| 2 | Expand High Fidelity PCR System, PCR buffer, 10x conc. with $MgCl_2$ | PCR buffer with 15 mM $MgCl_2$. | 11 732 641 001 | 1 vial, 1 ml |
| | | | 11 732 650 001 | 2 vials, 1 ml each |
| | | | 11 759 078 001 | 10 vials, 1 ml each |
| 3 | Expand High Fidelity PCR System, PCR buffer, 10x conc. without $MgCl_2$ | PCR buffer without $MgCl_2$. | 11 732 641 001 | 1 vial, 1 ml |
| | | | 11 732 650 001 | 1 vial, 1 ml |
| | | | 11 759 078 001 | 10 vials, 1 ml each |
| 4 | Expand High Fidelity PCR System, $MgCl_2$ 25 mM Stock Solution | To adjust final $Mg^{2+}$ concentration. | 11 732 641 001 | 1 vial, 1 ml |
| | | | 11 732 650 001 | 1 vial, 1 ml |
| | | | 11 759 078 001 | 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 | Store at −15 to −25°C. |
| 2 | PCR buffer, 10x 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 | PCR buffer, 10x conc. without $MgCl_2$ | |
| 4 | $MgCl_2$ 25 mM Stock Solution | Store at −15 to −25°C. |

## 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 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*
- Mineral oil (optional)

## 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.

  *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.*

- 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, high fidelity, and high specificity from all types of DNA.

  *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 $Mg^{2+}$.***

### 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)

### 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: 0.5 to 5 U/50 µl. A concentration of 2.6 U (0.75 µl) will usually produce satisfactory results.
- 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.
- dNTP concentration: Always use equal concentrations of all four dNTPs. The final concentration of each dNTP should be between 50 and 500 µM; the most commonly used concentration is 200 µM. If you increase the dNTP concentration, you must also increase the $Mg^{2+}$ concentration.

#### 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 during the reaction setup. If you are setting up multiple reactions, the volume of each master mix should be 10% greater than that required for the total number of PCR assays.

#### Preparation of master mix 1

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:

| 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 | variable | 300 nM |
| Reverse primer 2 | variable | 300 nM |
| Template DNA | variable | 0.1 – 250 ng (10 – 250 ng human genomic DNA, 0.1 – 15 ng plasmid DNA) |
| **Final Volume** | **25** | |

3. Mix and centrifuge briefly.

#### Preparation of master mix 2

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:

| Reagent | Volume [µl] | Final conc. |
|---|---|---|
| Water, PCR Grade* | 19.25 | – |
| PCR buffer, 10x conc. with $MgCl_2$ (Vial 2) | 5 | 1x (1.5 mM $MgCl_2$) |
| Enzyme Mix (Vial 1) | 0.75 | 2.6 U/reaction |
| **Final Volume** | **25** | |

*When titrating the $Mg^{2+}$ concentration, use the PCR buffer, 10x conc. without $MgCl_2$ (Vial 3) and the $MgCl_2$ 25 mM Stock Solution (Vial 4).*

3. Mix and centrifuge briefly.

## PCR protocol

*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.
   *Overlay the reaction carefully with mineral oil if required by the thermal cycler.*

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.

*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<br>Annealing<br>Elongation | 94<br>45 – 65[1]<br>72[2] or 68 | 15 sec<br>30 sec<br>45 sec – 8 min[3] | 10 |
| Denaturation<br>Annealing<br>Elongation | 94<br>45 – 68[1]<br>72[2] | 15 sec<br>30 sec<br>45 sec – 8 min[3] + 5 sec cycle elongation for each successive cycle[4] | 15 – 20 |
| Final Elongation | 72[2] | 7 min | 1 |
| Cooling | 4 | indefinitely | |

[1] Optimal annealing temperature depends on the melting temperature of the primers and on the experimental system.
[2] For PCR products up to 3 kb, elongation temperature should be +72°C; for PCR products >3 kb, elongation temperature should be +68°C.
[3] 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.
[4] 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

### 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*.

- For generating probes for Southern analysis, use a concentration of 50 µM modified dUTP (with 150 µM dTTP).
- When using Fluorescein-dUTP, increase the $MgCl_2$ concentration to 4 mM.
- For ELISA based detection systems, a concentration of 10 µM 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/µl

### Working Concentration

0.5 to 5 U per 50 µl reaction (optimal).
2.6 U (0.75 µl) per 50 µl 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:<br>▪ Analyze an aliquot on an agarose gel to check for possible degradation.<br>▪ Include a control reaction using a known template under established PCR conditions.<br>▪ 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 1.5 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 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 $MgCl_2$ 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.** | |
| ①②③ etc. | Stages in a process that usually occur in the order listed. |
| ❶❷❸ 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.

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