# **illustra** Hot Start Master Mix

## Product booklet

cytiva.com

29474447 AB

# Table of Contents

# 1 Introduction

### Product code

25150001 (100 reaction pack.)

### Important

Read these instructions carefully before using the products.

### Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

### Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

### Storage

Store at -20°C.

### Expiry

For expiry date please refer to outer packaging label.

# 2 Components

### Kit contents

### Hot Start Master Mix Formulation (2x)

Tris-HCl

KCl

$MgCl_2$

dNTPs (dATP, dCTP, dGTP, dTTP)

Taq DNA Polymerase

Hot Start Activator protein

Stabilizers

Additional $MgCl_2$ may be added for optimizing PCR conditions. Hot Start Master Mix (2x) combines Taq DNA Polymerase with a recombinant Hot Start Activator protein in a unique buffer formulation. Magnesium and nucleotide concentrations are at 3 mM and 0.4 mM each, respectively.

## Other materials required

### Reagents

Water—Use only deionized or distilled water that is sterile and free of contaminating nucleic acid.

DNA template

Primers (template specific)—Guidelines for primer design and use are described *Chapter 5 General considerations, on page 8*.

Mineral oil—if required for the thermal cycler being used.

### Equipment

Supplies for liquid handling—Gloves, vials, and sterile pipette tips; pipettor and microcentrifuge. Perform all reactions using sterile plastic tubes suitable for thermal cycling.

Thermal cycler—For cycling according to the specified conditions.

Ice bucket.

# 3 Description

The polymerase chain reaction (PCR), is a method for the *in vitro* amplification of a specific sequence of DNA. A typical PCR includes template DNA containing the target to be amplified, two primers that are complementary to the target DNA sequence, nucleotides, and a thermostable DNA polymerase. The reaction mixture is repeatedly cycled through alternating periods of thermal denaturation, annealing and extension, resulting in the exponential amplification of the target DNA sequence (1, 2, 3).

Hot Start PCR was developed as a method to eliminate nonspecific amplification and minimize primer-dimer formation during PCR. Both of these conditions can generate spurious products, background smears and generally reduce the overall efficiency and yield of PCR. Other hot start methods rely on maintaining the polymerase in an inactive state using monoclonal antibodies directed against the polymerase or by chemical modification of the enzyme itself (4, 5). Hot Start Master Mix utilizes a novel approach whereby primers are sequestered away from the polymerase using Hot Start Activator protein thus making them unavailable for nonspecific priming during reaction setup. The buffer is formulated to contain $MgCl_2$ but can be easily supplemented with additional $MgCl_2$ to meet specific needs.

# 4 Protocol

## Performing PCR with Hot Start Master Mix

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the master mix. For multiple reactions, scale up the volume of reaction components proportionally.

| Step | Action |
|---|---|
| 1 | Thaw reagents at room temperature. Mix thoroughly and then place on ice. |
| 2 | Assemble reactions on ice or at room temperature, whichever is more convenient. |
| 3 | The following table shows recommended component volumes: |

| Components | Volume for a 50 µL reaction | Final Concentration |
|---|---|---|
| Hot Start PC Master Mix (2x) | 25 µL | 1 x |
| Forward Primer | x µL | 0.1-0.5 µM |
| Reverse Primer | y µL | 0.1-0.5 µM |
| Template DNA[1] | z µL | as needed |
| Water | up to 50 µL | NA |

[1] Start with 50 pg for a simple template such as plasmid DNA, or 50 ng for a complex template such as genomic DNA. Avoid template amounts > 1 µg

| | |
|---|---|
| 4 | Mix reactions by pipetting or gentle vortexing then spin down briefly in a microcentrifuge. |

| Step | Action |
|---|---|
| 5 | Optional—Overlay reactions with one-half volume PCR-grade mineral oil if you are not using a heated lid on a thermal cycler. |
| 6 | The reactions are now ready for thermal cycling. |

## Thermal cycling

The optimal cycling profile for a given PCR system and thermal cycler will vary and must be determined empirically. Cycle number can range from 20–45 depending on the desired product yield. Thermal cycling results and product yield can vary with cycle conditions and the thermal cycler used. Read the instructions provided with your thermal cycler and optimize the reaction conditions accordingly.

Below is a general thermal cycling protocol:

| Step | Action |
|---|---|
| 1 | Initial denaturation.<br>Temperature: 94°C to 95°C.<br>Time: 2 min. |
| 2 | Denature.<br>Temperature: 94°C to 95°C.<br>Time: 30 s. |
| 3 | Anneal.<br>Temperature: 55°C.<br>Time: 30 s. |

| Step | Action |
|---|---|
| 4 | Extend.<br>Temperature: 68°C to 72°C.<br>Time: 30-60 s. |
| 5 | Repeat previous three cycles as necessary (steps 2-4), generally 20–45 times. |
| 6 | Final Extend.<br>Temperature: 68°C to 72°C.<br>Time: 5 min. |
| 7 | Final Soak.<br>Temperature: 4°C to 10°C.<br>Time: as necessary. |

# 5 General considerations

## Avoiding nucleic acid contamination

Adherence to some simple precautions will prevent nucleic acid contamination of reactions. Use sterile pipette tips with filters for dispensing, use autoclaved molecular biology grade water (or better) for reaction set up. In addition, work in a laminar air flow hood or PCR bubble that has been illuminated with UV light. It is important to analyze or pipette PCR products in an area separate from where the reaction will be set up.

To minimize environmental contamination, routinely treat all work surfaces with a 10% (v/v) bleach solution. Ideally, allow the bleach to contact the surface for at least 10 minutes prior to wiping away with sterile water.

## Primers and templates

In general, PCR primers should be 15–30 bp in length with a GC content of ~ 50%. Complementarity between primer pairs and within each primer should be avoided. There are a number of excellent Web sites for designing primers—some of our favorites are: *www.oligo.net* and *https://bioinfo.ut.ee/primer3-0.4.0/*

Template DNA purified by a variety of methods may be suitable substrates for PCR, but high quality DNA produces the most reproducible results. A typical PCR reaction contains < 1 µg of template DNA and primers at a concentration of 0.2–1.0 µM. The optimal quantity of template and primers must be determined empirically for each new combination of template and primer. The reaction conditions described in this protocol are general recommendations only.

## Primer annealing temperatures

The annealing temperatures of the chosen primers often vary from their estimated melting temperatures ($T_m$). Although amplification is more specific at higher annealing temperatures, there might be some loss of longer target sequences during amplification and an overall reduction in yield. On occasion, it is necessary to re-optimize annealing temperatures to achieve maximum performance. We recommend altering the temperature in increments of 2.5°C, starting at 10°C below the original annealing temperature.

## Magnesium chloride concentration

In the final reaction volume of 50 µL, the $MgCl_2$ concentration is 1.5 mM. If higher concentrations of $Mg^{2+}$ are desired, the following table can be used to determine the volume of sterile 10 mM $MgCl_2$ solution that should be added to increase the $Mg^{2+}$ concentration of the reaction. If $MgCl_2$ is added to the reaction, decrease the amount of water added to the reaction mixture to maintain a final reaction volume of 50 µL.

| Final [$MgCl_2$] | Volume of 10 mM $MgCl_2$ to add |
|---|---|
| 2.0 mM | 2.5 µL |
| 2.5 mM | 5 µL |
| 3.0 mM | 7.5 µL |
| 3.5 mM | 10 µL |
| 4.0 mM | 12.5 µL |
| 4.5 mM | 15 µL |
| 5.0 mM | 17.5 µL |

# 6 Troubleshooting guide

## Problem: No amplification

| Possible cause | Suggestions |
|---|---|
| *The thermal cycler did not function properly* | Improper cycling conditions can result in poor amplification. Consider testing a prevalidated PCR reaction as a control to produce a specific, known product. |
| *Primer was omitted from the reaction* | Hot Start Master Mix does not contain primers; you must add your own primers to the reaction mixture. |
| *The reaction volume was incorrect* | The total volume of each reaction should be twice the amount of Hot Start Master Mix added. |
| *Insufficient DNA was used in the PCR* | The amount of DNA required to generate product can vary between different PCR systems. Titer the amount of template in the reaction, starting with 50 pg for a simple template such as plasmid DNA, or 50 ng for a complex template such as genomic DNA. |
| *The quality of the DNA template was poor* | Impure DNA might fail to amplify. To inactivate contaminating enzyme activities, heat the template DNA to 95°C for 5 min before use in PCR. Alternatively, isolate template DNA by another method. |

## Problem: Excessive back ground amplification

| Possible cause | Suggestions |
|---|---|
| *Too much template DNA was added to the reaction* | Reduce the amount of template DNA in the reaction until the smearing is eliminated. |
| *Too many cycles* | Although the yield of PCR product can be increased by increasing the number of cycles to 45, this can produce spurious bands and increased background. Reduce the number of cycles until the smearing is eliminated. |
| *Kit storage* | If stored improperly the Hot Start Activator protein may be rendered inactive leading to decrease in hot start capability and increased background amplification. Always ensure proper storage of Hot Start Master Mix (2x). |
| *Cycling conditions vary depending on the thermal cycler used* | Optimize cycling conditions based on the manufacturer's recommendations. |
| *The annealing temperature was too low* | The optimal annealing temperature depends on the sequence of the primers and their homology to the template DNA. Re-optimization of annealing temperature might be required. Increase the annealing temperature by 2.5°C increments. |
| *The quality of the DNA template was poor* | Impure DNA can fail to amplify properly. Use freshly prepared DNA or isolate the template by another method. |

## Problem: Nonspecific amplification

| Possible cause | Suggestions |
|---|---|
| *Contamination in primers, template or buffers* | Prepare fresh materials. |

# 7 Related products

## dNTPs for PCR and Long PCR, Premixed

- Premixed dNTPs available in four concentrations specially formulated for PCR and long PCR.
- dNTPs functionally tested in PCR and in long PCR (14.4 kb) to be PCR-qualified.
- Packaged at the indicated concentration as the nucleoside triphosphate sodium salt.

| Product | Pack size | Product code |
|---|---|---|
| DNA Polymerization mix<br>(20 mM each dATP,dCTP,dGTP,dTTP) | 10 µmol | 27209401 |
| DNA Polymerization mix<br>(20 mM each dATP,dCTP,dGTP,dTTP) | 40 µmol<br>(4 x 0.5 mL) | 27209402 |
| PCR nucleotide mix<br>(25 mM each dATP,dCTP,dGTP,dTTP) | 500 µL | US77119-500UL |
| PCR nucleotide mix<br>(2 mM each dATP,dCTP,dGTP,dTTP) | 1 mL | US77170 |
| PCR nucleotide mix<br>(10 mM each dATP,dCTP,dGTP,dTTP) | 500 µL | US77212-500UL |
| dATP, dCTP, dGTP, dUTP Mix<br>(2 mM each dATP,dCTP,dGTP,4 mM dUTP) | 250 µL | US77175-250UL |

## PuReTaq Ready-To-Go PCR Beads

- Newly designed, premixed, predispensed reactions for PCR featuring high-performance PuReTaq DNA polymerase.

- Preformulated, predispensed, single-dose, ambienttemperature-stable beads ensure greater reproducibility between reactions, minimize pipetting steps and reduce the potential for pipetting errors and contamination.
- Use of PuReTaq DNA polymerase and other high-purity reagents ensures that each bead is free of contaminating DNA.
- Optimized for standard PCR, each bead yields a reaction containing ~ 2.5 units of PuReTaq DNA polymerase, 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, stabilizers, and BSA.
- Validated for real-time PCR with MGB Eclipse™ Probe Systems.

| Product | Pack size | Product code |
|---|---|---|
| PuReTaq Ready-To-Go™ PCR Beads (0.2 mL tubes/plate) | 96 reactions | 27955701 |
| PuReTaq Ready-To-Go PCR Beads (0.2 mL tubes/plate) | 5x96 reactions | 27955702 |
| PuReTaq Ready-To-Go PCR Beads (0.5 mL tubes) | 100 reactions | 27955801 |
| PuReTaq Ready-To-Go PCR Beads (0.2 mL hinged tube with cap) | 96 reactions | 27955901 |

## FideliTaq™ PCR Master Mix

- For PCR requiring high-fidelity DNA polymerase activity.
- Convenient, ready-to-use mix reduces experimental variability.

- Optimized for long PCR products.

| Product | Pack size | Product code |
|---|---|---|
| FideliTaq PCR Master Mix (2x) | 100 reactions (125 units) | E71182 |
| FideliTaq PCR Master Mix Plus | 100 reactions (125 units) | E71183 |

## ExoSAP-IT™

- For rapid and efficient purification of PCR products.
- Features two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase, for the removal of unwanted deoxynucleotides and primers with no interference in downstream applications.
- One-tube, one-step method with no sample loss.
- Purified DNA is ready for immediate use in manual and automated sequencing.
- Eliminates spin columns to decrease time and expense to purify PCR product.
- Simple processing makes ExoSAP-IT ideal in robotics, replacing beads, filtrations, and plates.

| Product | Pack size | Product code |
|---|---|---|
| ExoSAP-IT | 100 reactions | US78200 |
| ExoSAP-IT | 500 reactions | US78201 |
| ExoSAP-IT | 2000 reactions | US78202 |

## MicroSpin™ G-25 Columns

- For rapid buffer exchange/desalting of PCR products and other DNAs in a volume of 10–100 µL using spin-column chromatography.
- Excellent for rapid purification of newly synthesized oligonucleotides > 10-mers in 100–150 µL of deprotection solution using spin-column chromatography.
- Convenient, prepacked with Sephadex™ G-25 DNA Grade and pre-equilibrated in distilled water containing 0.05% Kathon™ CG/ICP Biocide.
- Ready to use, requires less than 4 min from sample application to the collection of purified product.
- Tested in nickase, single and double-stranded exonuclease and RNase assays.
- Can also be used for desalting/buffer exchange of DNA and removal of unincorporated radionucleotides from end-labeled oligonucleotides (at least 10 bases in length) in a volume of 10–100 µL.

| Product | Pack size | Product code |
|---|---|---|
| MicroSpin G-25 Columns | 50 | 27532501 |

## MicroSpin S-300 HR Columns

- MicroSpin S-300 HR Columns: For rapid purification of PCR products (> 100 bp) from unincorporated primers (< 20-mers) and nucleotides using spin-column chromatography.
- Accommodates 25-50 µL for post-PCR clean-up prior to sequencing.

- Useful for purification of alkaline-denatured plasmid DNA prior to sequencing.
- Convenient: Prepacked with Sephacryl™ S-300 HR pre-equilibrated in TE buffer.
- Ready to use: Requires less than 4 min from sample application to collection of purified product.
- Tested in nickase, single- and double-stranded exonuclease and RNase assays.

| Product | Pack size | Product code |
|---|---|---|
| MicroSpin S-300 HR Columns | 50 | 27532501 |

## MicroSpin S-400 HR Columns

- MicroSpin S-400 HR Columns: For rapid purification of PCR products (> 200 bp) from unincorporated primers (< 32-mers) and nucleotides using spin-column chromatography.
- Accommodates 25-50 µL for post-PCR clean-up prior to cloning or a second amplification reaction or 51-100 µL for all other applications.
- Convenient: Prepacked with Sephacryl S-400 HR pre-equilibrated in TE buffer.
- Ready to use: Requires less than 4 min from sample application to collection of purified product.
- Tested in nickase, single- and double-stranded exonuclease and RNase assays.

| Product | Pack size | Product code |
|---|---|---|
| MicroSpin S-400 HR Columns | 50 | 27514001 |

# 8 References


1. Mullis, K.B. *et al. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol*. 51, 263-273 (1986).
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3. Saiki, R.K. *et al*. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230 1350-1354 (1985).
4. Kellogg, D.E. *et. al. Taq*Start Antibody: "hot start" PCR facilitated by a neutralizing monoclonal antibody directed against *Taq* DNA polymerase. BioTechniques, 16, 1134–1137 (1994).
5. Moretti, T. *et al.* Enhancement of PCR amplification yield and specificity using AmpliTaq Gold DNA polymerase. *BioTechniques*, 25, 716-22 (1998).

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29474447 AB V:6 11/2021