illustra Hot Start Mix Ready-To-Go

Product booklet

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1 Introduction

Product codes

28900646 (0.5 mL 100 Reaction Pack)

28900653 (0.2 mL 96 Reaction Pack)

28900654 (0.2 mL 5 × 96 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

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at ambient room temperature in the airtight foil pouch with the desiccant. Once opened, completely reseal the pouch, fold the sealed edge over several times, and seal with a clip. In high humidity environments, store unopened and resealed pouches in a desiccator to maximize product lifetime.

Expiry

With proper storage, this product is stable for at least 12 months from date of receipt.

2 Components

Kit contents

The Hot Start Mix Ready-To-Go™ beads are premixed, predispensed and complete reactions ready for performing hot start PCR amplifications. The beads contain recombinant PuReTaq DNA polymerase, dNTPs, buffer, stabilizers and Hot Start Activator protein. With the exception of primer and template, the convenient, ambient temperature-stable beads provide all the necessary reagents to perform 25 µL hot start polymerase chain reactions.

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 in *Primers and templates, on page 10*.
- **Mineral oil**—if required for the thermal cycler being used - available from Cytiva.

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.

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 (see reference 1, 2, and 3 in *Chapter 8 References, on page 20*).

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 amplicons, background smears and generally reduce the overall efficiency of PCR and yield of specific amplification product. 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 (see reference 4 and 5 in *Chapter 8 References, on page 20*). Hot Start Mix Ready-To-Go beads utilize 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 1.5 mM $MgCl_2$ but can be easily supplemented with additional $MgCl_2$ to meet specific needs.

4 Protocol

Performing PCR with Hot Start Mix Ready-To-Go

Preliminary preparations and general handling instructions

Please note that the beads contain buffers, dNTPs, enzyme, stabilizers, Hot Start Activator protein and BSA, all which have been pretreated to minimize contamination. Discard any beads that were accidentally dislodged from their respective containers.

Step	Action
1	**Prepare the Hot Start Mix Ready-To-Go beads as follows:** **a.** Remove the desired quantity of tubes from the foil pouch. Remove individual tubes from a strip of eight by cutting the plastic link between tubes with scissors. **b.** Examine these tubes to verify that a bead is visible at the bottom of each tube. The beads are screened by weight and appearance. Please discard any beads that appear substantially smaller or misshapen— an indication of moisture contamination. Please refer to the recommended storage conditions. **c.** If necessary, gently tap the tube against a hard surface to force each bead to the bottom of the tube.

Step	Action

d. Place the tubes into a container that allows easy access during your experiment. The beads are now ready for use.

2 **Hot start PCR with Hot Start Mix Ready-To-Go**

For general information concerning primer design and cycling parameters, refer to *Primers and templates, on page 10*.

When performing PCR amplifications, exercise extreme care to prevent nucleic acid contamination as described in *Avoiding nucleic acid contamination, on page 9*. Each PCR bead is designed for use in a 25 µL reaction volume (one PCR bead/tube).

When resuspened in a final volume of 25 µL, each reaction will contain 1.5 mM $MgCl_2$. Please refer to *Magnesium chloride concentration, on page 12*. if higher concentration of $MgCl_2$ is desired.

a. For each reaction, add the following to a tube containing a PCR bead:

NOTICE

Do not mix the tube contents until all the components (below) have been added to the tube containing the bead.

Step Action

Components	Volume for a 25 µL reaction	Final Concentration
Hot Start Mix Ready-To-Go Bead	1 bead	1 ×
Forward Primer	×µL	0.1-0.5 µM
Reverse Primer	y µL	0.1-0.5 µM
Template DNA[1]	z µL	as needed
Sterile High Quality Water	to a final volume of 25 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

b. Snap the caps (provided) onto the tubes, pushing down firmly to ensure a tight fit. Mix the tube contents by gently flicking the tube with a finger. Vortex gently and then centrifuge the tube for a few seconds to bring the contents to the bottom of the tube. The reaction is fully dissolved and mixed when it appears clear.

c. 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.

Table 1. General thermal cycling protocol

Cycle Step	Temperature	Time
Initial denaturation	94–95°C	2 min
Denature	94–95°C	30 s
Anneal	55°C	30 s
Extend	68–72°C	30-60 s
Repeat previous three cycles as necessary, generally 20–45 times		
Final Extend	68–72°C	5 min
Final Soak	4–10°C	as necessary

5 General Considerations

Avoiding nucleic acid contamination

Hot Start Mix Ready-To-Go beads have passed rigorous quality tests to ensure the lowest possible levels of contaminating prokaryotic and eukaryotic nucleic acids. Adherence to some simple precautions will prevent the reintroduction of contamination. Use sterile pipette tips with filters for dispensing, select micro biology grade water (or

better) to formulate all buffers, and whenever possible, autoclave all buffers prior to use. 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 reactions will be set up.

To minimize environmental contamination with amplified products, 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 *http://frodo.wi.mit.edu*

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 optimal annealing temperature depends on the sequence of the primers and their homology to the template DNA. 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.

Magnesium chloride concentration

In the final reaction volume of 25 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 25 µL.

Final [$MgCl_2$]	Volume of 10 mM $MgCl_2$ to add
2.0 mM	1.25 µL
2.5 mM	2.75 µL
3.0 mM	3.75 µL
3.5 mM	5.00 µL
4.0 mM	6.25 µL
4.5 mM	7.50 µL
5.0 mM	8.75 µL

6 Appendix

Troubleshooting guide

Table 2. 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.

Possible cause	Suggestions
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	Each reaction should be at a final reaction volume of 25 µL.
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.
Examine beads for size and uniformity	If the pouch was previously opened and the beads subsequently stored without properly resealing the pouch, atmospheric moisture will rehydrate the beads. The result will be beads that are misshapen or markedly smaller. Reactions containing such beads should be discarded. To achieve optimum performance and maximum storage lifetime, store the beads in a dessicator after resealing the pouch by folding over the opened edge several times and clipping shut with a paper clip or the equivalent.

Table 3. 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.
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. With the Ready-To-Go bead format, annealing temperatures might vary slightly from those used in standard PCR master mixes. 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.
Too much primer was added to the reaction, resulting in the formation of primer-dimer bands	Excessive primer-to-template ratio can cause an abundance of low molecular weight bands and smearing even when using a hot Start PCR system. Titrate the amount of primers in the reaction until the primer-dimer band is eliminated.

Possible cause	Suggestions
Primers were not properly designed	Complementarities between primer pairs and within each primer should be avoided.

Table 4. Problem: Nonspecific amplification

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

7 Related products

illustra 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 bePCR-qualified.
- Packaged at the indicated concentration as the nucleosidetriphosphate sodium salt.

Product	Pack size	Product Code
DNA Polymerization mix (20 mM each dATP, dCTP, dGTP, dTTP)	10 µmol	28406557
DNA Polymerization mix (20 mM each dATP, dCTP, dGTP, dTTP)	40 µmol (4 × 0.5 mL)	28406558
PCR nucleotide mix (25 mM each dATP, dCTP, dGTP, dTTP)	500 µL	28406560
PCR nucleotide mix (2 mM each dATP, dCTP, dGTP, dTTP)	1 mL	28406562
PCR nucleotide mix (10 mM each dATP, dCTP, dGTP, dTTP)	500 µL	28406564

PuReTaq Ready-To-Go PCR Beads

- Premixed, predispensed reactions for PCR featuring highperformance PuReTaq DNA polymerase.

- Preformulated, predispensed, single-dose, ambient-temperaturestable 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.

Product	Pack size	Product Code
PuReTaqReady-To-Go PCR Beads (0.2 mL tubes/plate)	96 reactions	27955701
PuReTaqReady-To-Go PCR Beads (0.2 mL tubes/plate)	5 × 96 reactions	27955702
PuReTaqReady-To-Go PCR Beads (0.5 mL tubes)	100 reactions	27955801
PuReTaqReady-To-Go PCR Beads (0.2 mL hinged tube with cap)	96 reactions	27955901

Hot Start Master Mix

- For PCR requiring high specificity of amplification
- Eliminates non-specific PCR product by blocking > 99% Polymerase activity prior to thermal cycling
- Eliminates primer-dimer formation for maximum amplification efficiency

Product	Pack size	Product Code
illustra™ Hot Start Master Mix	100 reactions (125 units)	25150001

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 preequilibrated 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	27513001

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

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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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28900646PL AC V:9 02/2021