cytiva

illustra puReTaq **Ready-To-Go** PCR Beads

Product Booklet

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1 Introduction

Product codes

27955701 (0.2 ml tubes/plate of 96)

27955702 (0.2 ml tubes/5 plates of 96)

27955801 (0.5 ml tubes, 100 reactions)

27955901 (0.2 ml tubes, 96 reactions)

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 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 dessicator to maximize product lifetime.

2 Components of the kit

The newly developed puReTaq Ready-To-Go™ Polymerase Chain Reaction (PCR) Beads are premixed and predispensed complete reactions for performing PCR amplifications. The beads contain recombinant puReTaq DNA polymerase, an industry leader for ambient stability and purity. With the exception of primer and template, the convenient, ambient temperature-stable beads provide all the necessary reagents to perform 25-µL polymerase chain reactions. The beads are available predispensed into either 0.2-mL thin-walled micro-well plates (sufficient for 96 reactions), 0.2-mL thin-walled tubes or 0.5-mL PCR tubes.

The following components are included in this product:

puReTaq Ready-To-Go PCR Beads: Room temperature-stable beads containing stabilizers, BSA, dATP, dCTP, dGTP, dTTP, ~2.5 units of puReTaq DNA polymerase and reaction buffer. When a bead is reconstituted to a 25 µL final volume, the concentration of each dNTP is 200 µM in 10 mM Tris-HCl, (pH 9.0 at room temperature), 50 mM KCl and 1.5 mM $MgCl_2$.

Web address: *cytiva.com*

Visit the Cytiva home page for regularly updated product information.

3 Materials not supplied

Reagents

- **Water** - Use only deionized or distilled water that is sterile and free of contaminating nucleic acid.
- **DNA template** - Best results are obtained with high quality DNA, as discussed in *Primers and templates - general considerations, on page 10*.
- **Primers (template-specific)** - Guidelines for primer design and use are described in *Primers and templates - general considerations, on page 10*.
- **Mineral oil** - If required for the thermal cycler being used.

Equipment

- **Supplies for liquid handling** - Gloves, vials, and pipette tips should be sterile; pipettor and microcentrifuge. Perform all reactions in the plastic microcentrifuge tubes provided in the kit; these tubes are suitably sterile for thermal cycling.
- **Ice bucket or cold block** - For maintaining puReTaq PCR beads at 4°C during rehydration and prior to thermal cycling.
- **Thermal cycler** - For cycling according to the specified conditions.

4 Description

PCR, or Polymerase Chain Reaction, is a method for the *in vitro* amplification of a specific sequence of DNA. A typical PCR contains template DNA containing the target sequence to be amplified, two primers that are complementary to the target DNA sequence, nucleotides, and a thermal-stable DNA polymerase. The reaction mixture is repeatedly cycled through alternating periods of thermal denaturation, annealing, and extension, resulting in exponential amplification of the target DNA sequence (1,2,3). puReTaq Ready-To-Go PCR Beads are designed and manufactured using a proprietary technology licensed to Cytiva. Optimized specifically for PCR, the Ready-To-Go reaction beads are formulated with high quality buffer, nucleotides (dNTPs), and recombinant puReTaq DNA polymerase - only template DNA and template-specific primers need to be added. The Ready-To-Go PCR bead format significantly reduces the number of pipetting steps, thereby decreasing handling errors and increasing reproducibility.

5 Protocols

Avoiding nucleic acid contamination

puReTaq PCR 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 molecular biology grade water (or better) to

formulate all buffers, and wherever 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 that 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.

Preliminary preparations and general handling instructions

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

Prepare the PCR beads as follows:

Step	Action
1	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.
2	Examine these tubes to verify that a bead is visible at the bottom of each tube. The beads are carefully 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.

Step	Action
3	If necessary, gently tap the tube against a hard surface to force each bead to the bottom of the tube.
4	Place the tubes into a container that allows easy access during your experiment. The beads are now ready for use.
5	Proceed with the next part of the protocol.

PCR with Ready-To-Go PCR Beads

For general information concerning primer design and cycling parameters, refer to *Chapter 6 Appendix, on page 10*.

When performing PCR amplifications, exercise extreme care to prevent DNA contamination as described above. Each PCR bead is designed for use in a 25 µL reaction volume (one PCR bead/tube).

When resuspended in a final volume of 25 µL, each reaction will contain 1.5 mM $MgCl_2$. Please refer to *Magnesium chloride concentration, on page 11* if a higher concentration of $MgCl_2$ is desired.

Step	Action
1	For each reaction, add the following to a tube containing a PCR bead: ***Note:*** *Do not mix the tube contents until all the components (below) have been added to the tube containing the bead.*

Step Action

5′ (forward) primer (5–25 pmol)	X µL
3′ (reverse) primer (5–25 pmol)	Y µL
Template DNA[1]	Z µL
Sterile high-quality water	to a final volume of 25 µL

[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.

2 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 components to the bottom of the tube. The reaction is fully dissolved and mixed when it appears clear.

3 Place the reaction mixtures on ice or in a cold block until ready for cycling. Minimize the time on ice prior to cycling to prevent formation of background reaction products.

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 to 40 depending on the desired yield of product. Thermal cycling results and product yield can vary with cycle conditions and thermal cycler used. Read the instructions provided with your thermal cycler and optimize reaction conditions accordingly.

6 Appendix

Primers and templates - general considerations

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 must be avoided to minimize the production of primerdimers. There are a number of excellent Web sites for designing primers - two of our favorites are: *http://www.oligo.net* and *http://www.abrf.org*

Template DNA purified by a variety of methods is a suitable substrate for PCR, but high quality DNA produces the most reproducible results. A typical PCR 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. With the Ready-To-Go PCR bead format, annealing temperatures slightly higher than those used in standard reactions should be used if nonspecific products are present following amplification. 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. In contrast, when lower annealing temperatures are used, nonspecific amplification often increases along with an increase in specific target

amplification. On occasion, it is necessary to reoptimize annealing temperatures to achieve maximum performance. We recommend altering the temperature in increments of 2°C to 5°C, starting at 10°C below the original annealing temperature.

Magnesium chloride concentration

When each PCR bead is rehydrated in a reaction volume of 25 µL, the mixture will contain 1.5 mM $MgCl_2$. If a higher concentration of Mg^{2+} is desired, the following table can be used to determine the volume of a 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 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	2.50 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

Thermal cycling parameters

A standard three-step endpoint PCR cycling protocol consists of multiple cycles of denaturation (95°C), annealing (40–60°C) and extension (72°C). An initial denaturation step (95°C for 5 minutes) is recommended to ensure complete denaturation of the template DNA. In some cases, it may be possible to

amplify a target sequence using a two-step PCR where the denaturation step (95°C) is followed by a combined annealing/elongation step (50–65°C). For most standard, three-step PCRs, 35 cycles produces a 105–109-fold amplification of the target sequence. PCR product yield can be improved by increasing the number of cycles to 40.

7 Troubleshooting

Problem	Possible causes/solutions
1. No amplification	• The thermal cycler did not function properly. Improper cycling conditions can result in poor amplification. Consider testing PCR beads in a control reaction with template DNA and primers previously shown to work successfully. • Primer was omitted from the reaction. PCR beads contain no primer; they must be added to the reaction by the researcher. • The reaction volume was incorrect. Each PCR bead should be resuspended to a final reaction volume of 25 µL. Further dilution of the beads will severely compromise performance. Volumes less than 25 µL will alter salt concentrations and alter primer annealing stringency. • Insufficient DNA was used in the PCR. The amount of DNA required to generate good signal can vary between different PCR systems. Titrate 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.

Problem	Possible causes/solutions
	• 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 minutes 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.

Problem	Possible causes/solutions
2. Excessive background amplification	• Too much template DNA was added to the reaction. Reduce the amount of template DNA in the reaction until the smearing is eliminated. • The reaction utilized more than 35 cycles. Although the yield of PCR product can be increased by increasing the number of cycles to 40, 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 PCR bead format, annealing temperatures might vary slightly from those used in standard master mix PCR. Reoptimization of annealing temperature might be required. Increase the annealing temperature by 2°C to 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	Possible causes/solutions
	• Too much primer was added to the reaction, resulting in the formation of primerdimer bands. Excessive primer:template ratios can cause an abundance of low molecular weight bands and smearing. Titrate the amount of primers in the reaction until the primer-dimer band is eliminated. • Primers were not properly designed. Complementarity between primer pairs and within each primer should be avoided.
3. Nonspecific amplification	• The primers hybridized to a secondary site on the template. Design new primers that are less specific for the secondary site. Increase the annealing temperature by 2°C to 5°C increments until the desired specificity is achieved. • Contamination in primers, template, or buffers. Prepare fresh materials.

8 References

1. Mullis, K. B. *et al., Cold Spring Harbor Symp. Quant. Biol.* **51**, 263– 273 (1986).
2. Mullis, K. B. and Faloona, F. A., *Methods Enzymol*. **155**, 335–350 (1987).
3. Saiki, R. K. *et al., Science* **230** 1350–1354 (1985)

9 Companion products

Product	Pack size	Product code
Nucleic acid purification		
illustra™ tissue and cells GenomicPrep Mini Spin kit	50 reactions	28904275
illustra blood GenomicPrep Mini Spin kit	50 reactions	28904264
illustra Bacteria genomicPrep Mini Spin kit	50 reactions	28904258
illustra plasmidPrep Mini Spin kit	50 reactions	28904269
illustra TriplePrep kit (for RNA, DNA, and protein)	50 preparations	28942544
PCR Clean-Up		
illustra GFX™ PCR DNA and Gel Band Purification Kit	100 purifications 250 purifications	28903470 28903471
illustra GFX 96 PCR Purification Kit	10 × 96 well plates	28903445
Cloning		
Blunt-ended PCR Cloning Kit	40 reactions	RPN5110
Ready-To-Go T4 DNA Ligase	50 reactions	27036101

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For local office contact information, visit cytiva.com/contact

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