

Product Information

REDTaq® DNA Polymerase

Catalog Number **D4309**

Storage Temperature -20°C

TECHNICAL BULLETIN

Product Description

REDTaq DNA Polymerase is a unique blend of our high quality *Taq* DNA Polymerase combined with an inert red dye. The dye enables quick visual recognition of reactions to which enzyme has been added, as well as confirmation of complete mixing. The formulation allows aliquots (5-10 μL) from the PCR to be directly loaded onto an agarose gel without addition of electrophoresis loading buffers. The red dye migrates at the same rate as a 125 bp fragment in a 1% agarose gel. Because gel loading buffer is not added to the reaction mix, a sample can be re-amplified, such as in nested PCR.

The red dye has no effect on automated or manual DNA sequencing, ligase mediated ligations, or exonucleolytic PCR product digestion. Though exceptions may exist, the dye is generally inert in restriction enzyme digestions. If desired, the dye can be removed from the amplicon using any standard purification method.

The enzyme is provided at one unit/ μL for more accurate volume measurement and less waste. Reactions using REDTaq DNA Polymerase and its optimized 10 \times PCR buffer are formulated as any PCR mixtures. There are no additional reaction preparation steps or protocol changes required.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 min at 74°C .

Reagents Provided

- REDTaq DNA Polymerase, Catalog Number D5684
1 unit/ μL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, inert dye, 50% glycerol. Provided as 50, 250, 1,000 or 2,500 units
- 10 \times REDTaq PCR Reaction Buffer, Catalog Number B5926, 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM MgCl_2 and 0.1% gelatin. Provided as 1 ml package

Reagents and equipment required but not provided

- Deoxynucleotide Mix, Catalog Number D7295
10 mM each dATP, dCTP, dGTP, and TTP
- Water, PCR Reagent, Catalog Number W1754
- Mineral Oil, Catalog Number M8662 (optional)
- Primers
- DNA to be amplified
- Dedicated pipettes
- PCR pipette tips
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Catalog Numbers P3114 and P3364
- Thermal cycler

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage

Store all components at -20°C .

Procedure

Note: REDTaq 10 \times PCR Reaction Buffer has been formulated to be compatible with REDTaq DNA Polymerase. If other buffers are to be used, they must be formulated to account for 0.4 mM magnesium being added to the PCR from the enzyme. In this case the final enzyme concentration in the PCR is assumed to be 0.05 units/ μL . Other enzyme concentrations may require further magnesium concentration optimization. Optimal concentrations of template DNA, MgCl_2 , KCl, and PCR adjuncts, as well as pH, are often target specific. It may be necessary to determine the optimal concentration of each component.

1. Add the following reagents to a thin-walled 200 μ L or 500 μ L PCR tube in the order listed below.

| Amount | Component | Final Concentration |
|-------------|--|-------------------------|
| 5 μ L | 10 \times REDTaq PCR Reaction Buffer | 1 \times |
| 1 μ L | Deoxynucleotide Mix, D7295 | 200 μ M (each dNTP) |
| - μ L | Primer | 0.1-0.5 μ M |
| - μ L | Primer | 0.1-0.5 μ M |
| 2.5 μ L | REDTaq DNA Polymerase | 0.05 unit/ μ L |
| - μ L | Template DNA (typically 10 ng) | 200 pg/ μ L |
| q.s. | Water (Cat. # W1754) | |
| 50 μ L | Total volume | |

Note 1: A master mix is highly recommended when setting up multiple reactions.

Note 2: 10 \times REDTaq PCR Reaction Buffer should be vortexed vigorously prior to use.

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Add 50 μ L of mineral oil to the top of each tube to prevent evaporation if not using a thermal cycler with a heated lid.
4. Optimum cycling parameters vary with PCR composition (i.e. primer sequences, template, MgCl₂ concentration etc.) and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximum product yield and/or quality. Typical cycling parameters are:

| For cycles 1-30 | | |
|-----------------|-----------------|-------|
| Denaturation | 94 $^{\circ}$ C | 1 min |
| Annealing | 55 $^{\circ}$ C | 2 min |
| Extension | 72 $^{\circ}$ C | 3 min |

Note: 25-30 cycles of amplification are recommended.

5. The amplified DNA can be evaluated by agarose gel electrophoresis by loading 5-10 μ L of the PCR reaction onto the gel without the addition of gel loading buffers.

Note: A minimum of 1.5 units of REDTaq DNA polymerase must be added per 50 μ L reaction for unencumbered gel loading. The red dye migrates as a 125 bp fragment in a 1% agarose gel. If a more intense tracking dye is desired, an unused lane can be used to run any common tracking dye (i.e. as provided by a DNA marker). Alternatively, a tracking solution devoid of DNA can be added to a previously loaded REDTaq PCR product well. Amplification products can be visualized by standard methodologies (e.g. ethidium bromide staining). Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase. Alternatively, an aliquot of the aqueous phase can be taken by withdrawing solution from below the aqueous phase/mineral oil interface.

Related Products

Reagents and Kits

- Lambda Hind III DNA Marker, Catalog Number D9780
- Enhanced Avian HS RT-PCR kits, Catalog Number HSRT100 (100 reactions). The RT-PCR kit combines two powerful techniques to convert mRNA into cDNA and subsequently to amplify the cDNA. The enhanced avian reverse transcriptase has an enhanced ability to transcribe through difficult secondary structure at elevated temperatures (up to 65 $^{\circ}$ C). Includes JumpStart AccuTaq™ LA for hot-start PCR.

Equipment

- PCR Multiwell Plate, 96-well, Catalog No. Z374903
- PCR Multiwell Plate, 384-well, Catalog No. Z374911
- PCR Microtubes, 0.2 ml, attached caps, Catalog No. Z374873
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Catalog No. Z374962
- Sealing accessory for PCR vessels, Micro Mats, Catalog No. Z374938
- PCR Workstation, 120V, Catalog No. Z376213
- PCR Workstation, 240V, Catalog No. Z376221

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Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: US 8,404,464 and US 7,972,828. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims.

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