



## Product Information

### REDTaq™ DNA Polymerase

Recombinant, expressed in *E. coli*

Product Code **D 4309**

Technical Bulletin No. MB-535

Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

REDTaq™ DNA Polymerase is a unique blend of Sigma's 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 per microliter for more accurate volume measurement and less waste. Reactions using REDTaq and its optimized 10X 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, Product Code D 5684  
1 unit/µl in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% Igepal CA-630, inert dye, 50% glycerol. Provided as 50, 250, 1,000 or 2,500 units
- 10x REDTaq PCR Reaction Buffer, Product Code B 5926, 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM MgCl<sub>2</sub> and 0.1% gelatin. Provided as 1 ml package

### Reagents and Equipment Required but Not Provided

- Deoxynucleotide Mix, Product No. D 7295  
10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP
- Water, PCR Reagent, Product Code W 1754
- Mineral Oil, Product Code M 8662 (optional)
- Primers
- DNA to be amplified
- Dedicated pipettes
- PCR pipette tips
- 0.5 ml or 0.2 ml thin-walled PCR tubes, Product Codes P 3114 and P 3364
- Thermal cycler

### Precautions and Disclaimer

Sigma's REDTaq DNA Polymerase is for R&D use only. Not for drug, household or other uses. Refer to the Material Safety Data Sheet (MSDS).

### Storage

Store all components at –20 °C.

## Procedure

**Note: REDTaq 10X PCR Buffer has been formulated to be compatible with REDTaq. 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 per microliter (2.5 units per 50 microliter reaction). Other enzyme concentrations may require further magnesium concentration optimization.**

Optimal concentrations of template DNA, MgCl<sub>2</sub>, 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 µl or 500 µl PCR tube in the order listed below.

Amount	Component	Final Concentration
5 µl	10X REDTaq PCR Buffer	1 X
1 µl	Deoxynucleotide Mix, D 7295	200 µM (each dNTP)
- µl	Primer	0.1-0.5 µM
- µl	Primer	0.1-0.5 µM
2.5 µl	REDTaq DNA Polymerase	0.05 unit/µl
- µl	Template DNA (typically 10 ng)	200 pg/µl
q.s.	Water	
50 µl	Total reaction	

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

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.
3. Add 50 µl 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, MgCl<sub>2</sub> concentration etc.) and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximum product yield and/or quality. Common cycling parameters are:

### For cycles 1-30

Denaturation	94 °C	1 min
Annealing	55 °C	2 min
Extension	72 °C	3 min

25-30 cycles of amplification are recommended.

5. The amplified DNA can be evaluated by agarose gel electrophoresis by loading 5-10 µl 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 µl 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.

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