3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

REDTaq® ReadyMix™ PCR Reaction Mix with $MgCl_2$

Catalog Number **R2523**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

REDTaq® ReadyMix™ PCR Reaction Mix combines the performance and benefits of Sigma's REDTaq DNA polymerase with the convenience of a ReadyMix PCR Reaction Mix. REDTaq ReadyMix is a ready-to-use mixture of *Taq* DNA polymerase, 99% pure deoxynucleotides, reaction buffer, and an inert red dye in a 2× concentrate. It saves preparation time, reduces the risk of contamination from multiple pipetting steps, and provides consistent reaction-to-reaction performance.

After the PCR reaction, the PCR product can be loaded directly onto an agarose gel. There is no need to add a loading buffer/tracking dye prior to electrophoresis. The inert red dye migrates at approximately the same rate as a 125 base pair fragment in a 1% agarose gel. Because the dye has no effect on the amplification process, a sample can be easily reamplified such as in "nested PCR".

For a typical PCR reaction, mix 25 µL of REDTaq ReadyMix PCR Reaction Mix with 25 µL of a mixture containing template DNA, primers, and water. Reaction volumes can be scaled down, if desired.

Reagents provided

- REDTaq ReadyMix PCR Reaction Mix, with $MgCl_2$
  Catalog Number R2648
  20 mM Tris-HCl, pH 8.3, with 100 mM KCl, 3 mM $MgCl_2$, 0.002 % gelatin, 0.4 mM dNTP mix (dATP, dCTP, dGTP, TTP), stabilizers, and 0.06 unit/µL of *Taq* DNA Polymerase.
  Provided as 20 reactions or 5 × 20 reactions
- Water, PCR Reagent, Catalog Number W1754
  Provided as a 1.5 ml vial

Equipment and reagents required, not provided

- DNA template to be amplified
- Primers
- Mineral Oil, Catalog Number M8662 (optional)
- 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.

When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed. Refer to Material Safety Data Sheet.

Storage/Stability

Store at –20 °C. For short-term storage of one week or less, store at 2-8 °C, so there is no waiting for reaction components to thaw. Repeated freeze/thaw cycles do not affect the activity of the reactions.

Procedure

The optimal conditions for template DNA, primers, and cycling parameters will depend on the system being utilized.

Sigma offers a separate PCR Optimization Kit II, Catalog Number OPT2, that contains a variety of buffers and adjuncts for optimizing the specificity, fidelity, and yield of a PCR product.

1. Add the following reagents to a 0.2 or 0.5 ml PCR tube in the following order:

Volume	Reagent	Final Concentration
25 µL	REDTaq ReadyMix	1×
1 µL	Forward Primer	0.1–1.0 µM (15-30 bases in length)
1 µL	Reverse Primer	0.1–1.0 µM (15-30 bases in length)
X µL	Template DNA	
q.s.	Water	
50 µL	Total Volume	

2. Mix gently 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 using a thermal cycler without a heated lid.

4. The amplification parameters should be optimized for individual primers, template, and thermal cycler.

   Common cycling parameters:
   a. Denature the template at 94 °C for 1 minute
   b. Anneal primers at 55 °C for 2 minutes
   c. Extension at 72 °C for 3 minutes

   25-30 cycles of amplification are recommended.

5. The amplified DNA can be loaded directly onto an agarose gel after the PCR process. It is not necessary to add a separate loading buffer/tracking dye. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase. The red tracer co-migrates with 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.

AF,CR,PHC 09/10-1

Label License Statement

NOTICE TO PURCHASER: LIMITED LICENSE

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.