

REDiant II Taq DNA Polymerase (recombinant)

Cat. No.: BIO-5116-50U
BIO-5116-500U
BIO-5116-2500U

Concentration: 0.3U/ μ l

Supplied with: 10X Taq II buffer
25mM MgCl₂

Storage: -20°C.
Avoid frequent thawing and freezing.

Quality Control

Nuclease Assay: No detectable contaminating endonuclease or exonuclease activity.

Functional Assay: REDiant II Taq DNA Polymerase was tested for amplification of a 3500bp of human genomic DNA.

1.0 DESCRIPTION

REDiant II Taq DNA Polymerase is a thermostable DNA polymerase that catalyzes a 5'→3' polymerase activity and a 5' flap endonuclease activity. In addition, REDiant II Taq DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant REDiant II Taq DNA Polymerase is ideal for standard PCR templates up to 6kb. It includes a red, inert tracking dye which migrates at the same rate as 1kb DNA fragment in a 1% agarose gel. Inert dye does not inhibit PCR.

Source

An *E.coli* strain that carries a cloned gene from *Thermus aquaticus*.

Unit Definition

One unit of the enzyme catalyzes the incorporation of 10nmol of deoxyribonucleotides into an acid-insoluble material in 30mins at 74°C by using activated salmon sperm DNA as a template/primer.

Applications

- DNA sequencing
- DNA labeling
- PCR for cloning
- PCR amplification of DNA fragments up to 6kb
- Routine PCR

Buffer Composition

Storage buffer

| | |
|--------------------|------------|
| 20mM Tris-HCl | 0.1mM EDTA |
| Stabiliser | 1mM DTT |
| 50% (v/v) Glycerol | 100mM KCl |

10X Taq II buffer

750mM Tris-HCl (pH 8.8 at 25°C)
200mM KCl
50mM (NH₄)₂SO₄
0.5% (v/v) Nonidet P40

Remarks

- Half-life of this enzyme is >40mins @ 95°C.
- Taq II DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.

2.0 PROTOCOL

The following protocol serves as a starting point and general guideline for any Polymerase Chain Reaction (PCR). Reaction conditions (incubation times and temperatures, concentration of Taq II DNA polymerase, primers, MgCl₂ and template DNA) vary and need to be optimised. PCR reactions should be prepared in a DNA-free environment, dedicated pipette and aerosol resistant tips are recommended. Always keep the control and template DNA to be amplified isolated from other components.

Recommendations of Template DNA in a 50 μ l reaction volume

| DNA Type | Amount of DNA |
|---------------------------|------------------|
| Human genomic DNA | 0.1 to 1 μ g |
| Plasmid DNA | 0.5 to 5 ng |
| Phage DNA | 0.1 to 10 ng |
| <i>E.coli</i> genomic DNA | 10 to 100 ng |

1. Thaw REDiant II Taq DNA polymerase, 10X Taq II buffer and 25mM MgCl₂ at room temperature. Keep the tubes on ice after thawing. Vortex briefly and spin down contents quickly.
2. Prepare the following reaction mix in a sterile, nuclease-free PCR tube on ice.

Table 1. For a 50 μ l reaction volume:

| Components | Volume (μ l) | Final Concentration |
|-------------------------------|-------------------|---------------------|
| REDiant II Taq DNA Polymerase | 4.5 | 1.5U |
| Forward Primer (10 μ M) | 0.5 – 5 | 0.1 – 1 μ M |
| Reverse Primer (10 μ M) | 0.5 – 5 | 0.1 – 1 μ M |
| 10X Taq II Buffer | 5 | 1X |
| dNTP Mix (10mM of each) | 1 | 0.2mM |
| 25mM MgC ₂ | 2-6 | 1 - 3mM |
| DNA Template | 1-5 | See above |
| Nuclease-Free Water | Top up to 50 | N.A |

3. Cap tubes and spin down contents briefly.
4. Place reactions in thermal cycler and incubate at 95°C to completely denature template DNA. Perform 25 - 35 cycles of PCR amplification by repeating steps 2 to 4 in the PCR reaction. Low amounts of starting template may require 40 cycles.

Table 2. PCR Amplification as follows:

| Step | Temperature | Time |
|----------------------|-------------|-----------------|
| Initial Denaturation | 95°C | 1 – 5 min |
| Denaturation | 95°C | 0.5 – 1 min |
| Annealing | 50- 68°C | 0.5 – 1 min |
| Extension | 72 - 75°C | *0.5 – 1 min/kb |
| Final Extension | 72 - 75°C | 5 – 15 min |
| Soak | 4°C | ∞ |

* For amplification of >3kb, it is recommended to use 1min/kb as extension rate.

3.0 General guidelines for PCR amplifications

Initial denaturation

To ensure efficient utilization of the template during the first amplification cycle, it is essential that the template is denatured completely. If GC content of the template is \leq 50%, an initial 1-5 mins denaturation at 95°C would be sufficient.

Denaturation

DNA denaturation time of 0.5min/cycle at 95°C is usually sufficient. For GC-rich templates, denaturation could be prolonged to 3-4mins.

Annealing

Annealing temperature should be 5°C lower than melting temperature ($T_m - 5^\circ\text{C}$) of primers. 0.5min/cycle is usually sufficient. If non-specific PCR products are observed, the temperature should be optimized stepwise in 1-2°C increments.

Extension

Optimal temperature for extension of Taq II DNA Polymerase is between 70-75°C. Recommended extension step is 30sec/kb at 72°C for PCR products.

Number of cycles

If less than 10 copies of template are present, about 40 cycles are required. For a higher amount, 25-35 cycles are sufficient.

Final extension

After the last cycle, incubate PCR mixture at 72°C for an additional 5-15mins to allow any possible incomplete amplification to take place.