REDiant II PCR Master Mix (2X)

Cat. No.: BIO-5187-20
BIO-5187-200
BIO-5187-1000

Concentration: 60U/µl Taq II DNA polymerase (recombinant), 400uM dNTP mix, 3mM $MgCl_2$

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

Quality Control

Nuclease Assay: No detectable contaminating endonuclease or exonuclease activity.

Functional Assay: REDiant II PCR Master Mix (2X) was tested for amplification of a 3500bp of human genomic DNA.

1.0 DESCRIPTION

REDiant II PCR Master Mix (2X) is a premixed, ready to use solution which contain Taq II DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer for amplification of DNA templates by PCR. This pre-mixed formulation saves time and reduces contamination by reducing the number of pipetting steps required for usual PCR set up. The mix is optimised for efficient and reproducible PCR. It also includes a red, inert tracking dye which migrates at the same rate as 1kb DNA fragment in a 1% agarose gel.

This offers several advantages:

- Direct loading of PCR products onto agarose gel without addition of gel loading buffer
- Visualises the addition of polymerase into the PCR reaction mix and ensure complete mixing
- Acts as tracking dye in gel electrophoresis
- Red inert dye does not inhibit PCR

Source

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

Unit Definition

One unit of the enzyme catalyses 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

REDiant II PCR Master Mix (2X) Composition
Contains reaction buffer, 0.06U/ul of Taq II DNA polymerase, 3mM $MgCl_2$ and 400uM of each dNTPs.

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, $MgCl_2$ 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 µl reaction volume

DNA Type	Amount of DNA
Human genomic DNA	0.1 to 1 µg
Plasmid DNA	0.5 to 5 ng
Phage DNA	0.1 to 10 ng
E.coli genomic DNA	10 to 100 ng

1. Thaw REDiant II PCR Master Mix, 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 50µl reaction volume:

Components	Volume (µl)	Final Concentration
REDiant II PCR Master Mix (2X)	25	1.5U
Forward Primer (10µM)	0.5 – 5	0.1 – 1µM
Reverse Primer (10µM)	0.5 – 5	0.1 – 1µM
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 utilisation of the template during the first amplification cycle, it is essential that the template is denatured completely. If GC content of the template is ≤ 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°C) of primers. 0.5min/cycle is usually sufficient. If non-specific PCR products are observed, the temperature should be optimised 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 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.