BioReady Pfu pac.

Kit Components

Cat#	BSA10S1	BSA10M1
Components	**100units**	**250units**
10 × Reaction Buffer (with 20mM $MgSO_4$)	400 μ l	1000 μ l
BioReady Pfu （5U/μl）	80 μl	200 μl
2.5mM dNTP Mixture	300 μ l	500 μ l
6 ×loading dye	200 μ l	500 μ l
ddH_2O	1250 μ l	1250 μ l

Description:

BioReady Pfu is a thermostable enzyme of approximately 92 kDa isolated from *pyrococcus furiosus*. BioReady Pfu catalyzes the DNA-dependent polymerization of nucleotide into duplex DNA in the 5'→3' direction in the presence of magnesium ions. The enzyme also exhibits 3'→5'exonuclease (proofreading) activity. Base misinsertions that may occur infrequently during polymerization are rapidly excised by the proofreading activity of the polymerase. Consequently, BioReady Pfu is useful for polymerization reactions requiring high fidelity synthesis.

Ⅱ.Standard Application

A. PCR amplification

Reagent to be supplied by the user

* Sense primer
* Antisense primer
* DNA template

1. In a sterile, nuclease-free micro centrifuge tube, combine the following components:

10 x Buffer	5 μl
dNTP Mixture	4 μl
Sense primer (10 μM)	0.5-5 μl
Antisense primer (10 μM)	0.5-5 μl
BioReady Pfu	1.25units
Template DNA	<100ng
ddH_2O	up to 50 μl

Note: 1. **I**t is critical to withhold BioReady Pfu until the addition of dNTP; otherwise, the proofreading activity of the polymerase may degrade the primers, resulting in nonspecific amplification and reduced

product yield. Assemble components on ice.

2. If using a thermal cycler without hot lid, overlay the reaction mix with 1-2 drops (approximately 50µl) of the mineral oil to prevent evaporation during thermal cycling. Centrifuge the reaction mix in a micro centrifuge for 5 seconds.

3. Immediately place the reactions in a thermal cycler that has been preheated to 95℃ for 1-2 minutes to ensure that the target DNA is completely denatured. Incubation for longer than 2 minutes at 95℃ is unnecessary and may reduce the yield due to DNA damage.

4. Start the thermal cycling program. The cycling profile given in Table 1 may be used as a guideline. Optimize the amplification profile for each primer target combination.

Table 1. Recommended thermal cycling conditions for BioReady Pfu -mediated PCR amplification.

This guideline applies to target sequences between 200 and 2000bp and are optimal for comparable thermal cyclers.

Step	Temperature	Time	Number of Cycle
Initial denaturation	95℃	1-2 minutes	1 cycle
Denaturation	95℃	0.5-1 minute	
Annealing*	42-65℃	30seconds	25-35cycle
Extension**	72-74℃	2-4 minutes	
Final extension	72-74℃	5 minutes	1 cycle
Soak	4-8℃	indefinite	1 cycle

* The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers. See section Ⅲ for discussions on determining optimal annealing temperatures for PCR amplification.

** Allow approximately 2 minutes for every 1kb to be amplified.

Ⅲ. General Consideration

Enzyme consideration

We recommend that 1.25 units of BioReady Pfu be used per 50µl amplification reaction. The inclusion of more enzyme will increase 3'→5'exonuclease (proofreading) activity. It is essential to withhold BioReady Pfu from the reaction until after the addition of the dNTP mixture and to assemble components on ice.

Primer design

The sequences of the primers are a major consideration in determining the optimal temperature of the PCR amplification cycles. For primers with a high Tm, it may be advantageous to increase the annealing temperature. Higher temperature minimized nonspecific primer annealing, increase the amount of specific product and reduces the amount of primer-dimer formation.

The 3'→5' exonuclease activity may degrade primers. To overcome the degradation, longer primers with maximized GC content could be used. Primers can also be protected by introducing phosphothioate bonds at their 3' termini.

Extension time

The extension rate of BioReady Pfu is lower than of Taq DNA polymerase. Therefore, during the extension step, allow approximately 2 minutes for every 1kb to be amplified (minimum extension time of 1 minute). For most reaction, 25-35 cycles are sufficient.

References

1. Lundberg , K.S. et al (1991) High-fidelity amplification using a thermostable DNA polymerase isolated from pyrococcus furious.gene 108.1
2. Cline, J.Braman, J.C. and Hogrefe, H.H (1996) PCR fidelity of Pfu DNA polymerase and other thremostable DNA polymerase. Nucleic Acids Res. 24.3546.

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