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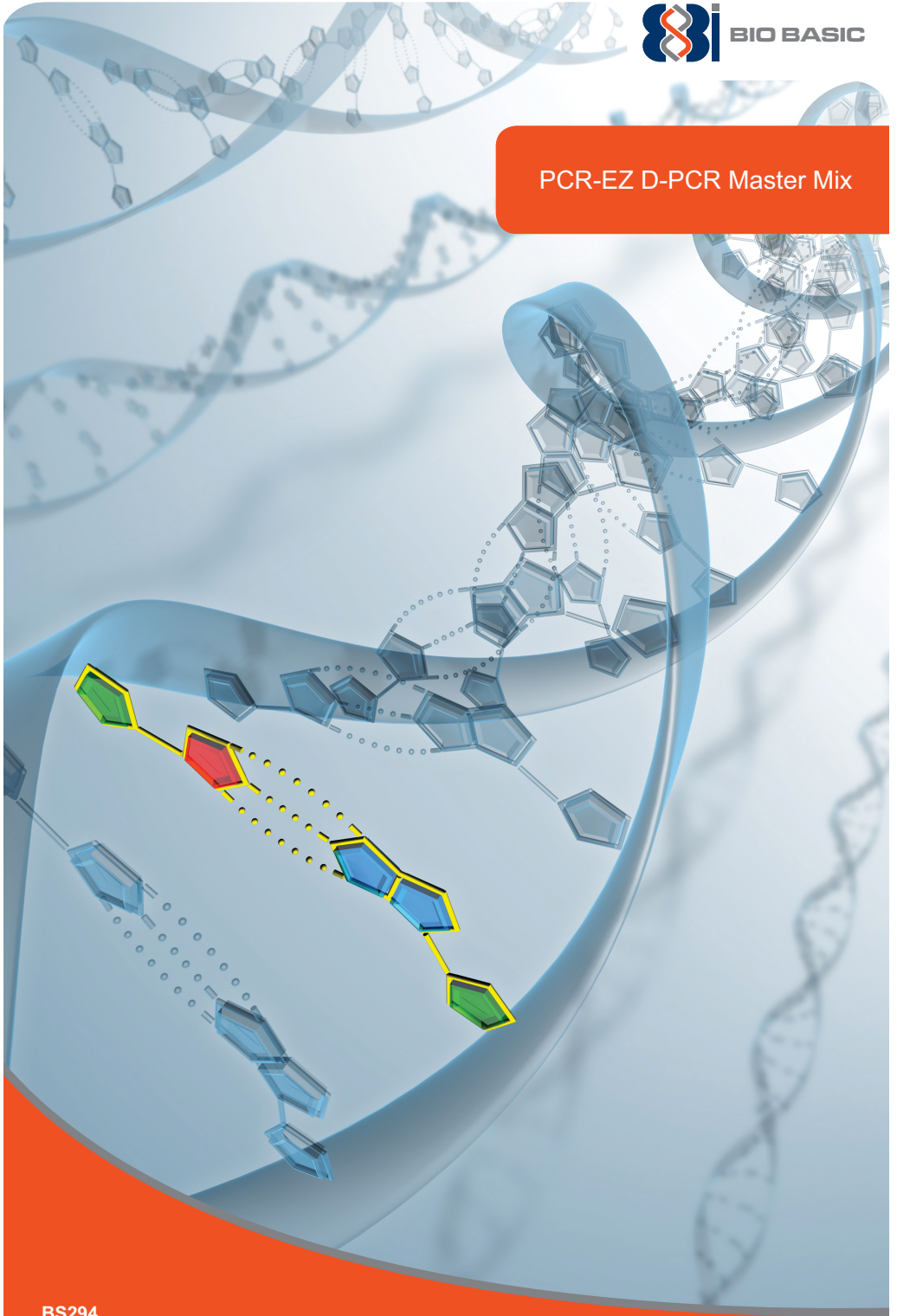
PCR-EZ D-PCR Master Mix



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For Research Use Only

PCR-EZ D-PCR Master Mix

SKU: BS294

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Description

The PCR-EZ D PCR MASTER MIX provides an efficient, accurate, and convenient way to amplify DNA from any template.

Taq DNA Polymerase is a thermostable DNA polymerase isolated from a strain of Thermus Aquaticus, and it has a half life of 3 hours at 95°C.

Taq has high fidelity with an error frequency of $10/10^6$(or $0.01/10^3$) during DNA synthesis. It is designed for use in primer extension reaction as well as sequencing. DNA sequencing with Taq DNA Polymerase produces uniform bands intensities and low background.

Taq DNA Polymerase is highly purified, free of contaminating endonucleases, exonucleases and nicking activity. For endonuclease assay, 1µg of Lambda / Hind III DNA is incubated with 20 units of the enzyme in assay buffer at 75°C for 16 hrs and no visible contaminating activity is observed.

For exonucleases assay, 1µg of pBR322 plasmid DNA is incubated with 10 units of enzyme for 16 hrs at 75°C in assay buffer and no detectable exonuclease is observed. The purity of the enzyme is also evaluated by adding 10 units of Taq DNA Polymerase in 100 µl of a reaction mixture for making first strand cDNA and no impaired effect on the first strand is observed.

Other Kits Available

EZ-500 Spin Column Plasmid DNA MaxiPreps Kit
BS465 (10preps), BS466 (20preps)

EZ-10 Spin Column Plasmid DNA MiniPreps Kit

EZ-10 Spin Column PCR Products Purification Kit

EZ-10 Spin Column DNA Gel Extraction Kit

And much more...

Nelson, K., Brannan, J. & Kretz, K., (1995) The fidelity of TaqPlus™ DNA Polymerase in PCR. Strategies Mol. Biol. 8:24–25.

Roux, K. H. (1995) Optimization and troubleshooting in PCR. PCR Methods Appl. 4:5185–5194.

Sambrook, J. & D. W. (2001) Molecular Cloning: A Laboratory Manual, Third Edition (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

List of Components

Store all components at -20°C. Enough reagents are supplied for a total of 5X20 PCR reactions.

	BS294 (5X20 PCR Reactions)
PCR EZ MASTER MIX [a) b)]	5 X 500µl
Control DNA Template(10ng/µl) [c)]	1X 100µl
5`-Control Primer I (10µM) [d)]	1X 20µl
3`-Control Primer II(10µM) [d)]	1X 20µl
PCR Grade Water	2 x 2ml

Each vial of PCR EZ MASTER MIX (500µl) is sufficient enough for 20 PCR reactions. Each PCR reaction requires 25µl from PCR EZ MASTER MIX.

a) 1 Unit of Taq Polymerase is used for each PCR Reaction.
b) Final concentration in each PCR reaction: 10mM KCl, 10mM $(NH_4)2SO_4$, 20mM Tris HCl (pH 8.75), 0.1% Triton X-100, 0.1mg/ml BSA, 2mM $MgCl_2$ and 200µM dNTPs.
c) The template is lamda DNA provided at 10ng/µl.
d) Control Primer Mix (10 µM each).

5′ primer 5′–ATA ACGCGTCGCCGGAAGCC –3′
3′ primer 5′–GACCGGGGAGGATACGTTTCAC–3

Additional materials required

The following reagents are not supplied.
– Thermal cycler
– Dedicated pipettors (1-2 µl, 1-10 µl, 1-20 µl, 20-200 µl, 200-1000 µl)
– PCR pipette tips suitable for use with the above pipettors and preferably equipped with hydrophobic filters
– DNA size markers
– 5X Stop/loading buffer
– PCR reaction tubes
– Mineral oil [Optional]: We recommend Bio Basic Inc. Cat. No. DBJ328

GENERAL CONSIDERATIONS

Amplification Size

Yields are unaffected when amplifying products less than 3-4 kb. For longer fragments, we recommend to increase the amount of Taq Polymerase slightly.

Good PCR Practices

1. Prepare reactions with dedicated pipettors in a dedicated work space

Due to the tremendous amplification power of PCR, any small amounts of contaminating DNA can produce nonspecific amplification; we recommend using small aliquots of starting material to avoid contaminating stocks.

When performing PCR, it is a good practice to wear gloves at all times and set up reactions in a dedicated lab area or noncirculating containment hood using dedicated pipettors, PCR pipette tips with hydrophobic filters, and dedicated solutions. We also recommend setting up a negative control reaction that does not contain any template.

2. Pipetting

We recommend using pipette tips with hydrophobic filters. Also be sure that no extra solution is on the outside of the pipette tip before transfer. When adding solution to a tube, immerse the tip into the reaction mixture, deliver the contents from the pipette tip into the mixture, and pipet up and down several times.

References

Barnes, W. M. (1994) PCR amplification of up to 35-kb DNA with high fidelity and high yield from λ bacteriophage templates. Proc. Natl. Acad. Sci. USA 91:2216–2220.

Cheng, S., Fockler, C., Barnes, W. M. & Higuchi, R. (1994) Effective amplification of long targets from cloned inserts and human genomic DNA. Proc. Natl. Acad. Sci. USA 91:5695–5699.

Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K. & Mattick, J. S. (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 19:4008.

Frey, B. & Suppmann, B. (1995) Demonstration of the Expand™ PCR system's greater fidelity and higher yields with a lacI-based PCR fidelity assay. Biochemica 2:8–9.

Kellogg, D. E., Rybalkin, I., Chen, S., Mukhamedova, N., Vlasik, T., Chenchik, A. & Siebert, P. (1994) TaqStart: A Neutralizing Monoclonal Antibody that facilitates “Hot Start” PCR. Clontechniques IX(2):1–5.

Longo, M. C., Berninger, M. S. & Hartley, J. L. (1990) Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 93:3749.

Too much enzyme
The Polymerase is suitable for most applications; however, a 1X final concentration of the enzyme mix may be too high for some applications. If smearing is observed, first try optimizing the cycle parameters as described above, then try reducing the enzyme concentration to 0.5–0.2X.

Too much template
Try a lower concentration of DNA template in the PCR reaction.

E. Dealing with contamination

Contamination most often results in extra bands or smearing. It is important to include a negative control (a control that replaces the DNA template with PCR-grade H2O but still includes the primers) in every PCR experiment to determine if the PCR reagents, pipettors or PCR reaction tubes are contaminated with previously amplified targets.

If possible, set up the PCR reaction and perform the post-PCR analysis in separate laboratory areas with separate sets of pipettors.

Laboratory benches and pipettor shafts can be decontaminated by depurination. Wipe surfaces with 1N HCl followed by 1N NaOH. Then neutralize with a neutral buffer (e.g., Tris or PBS) and rinse with ddH2O.

We advise using commercially available aerosol-free pipette tips.

3. Use a Master Mix

Assembling a Master Mix, which contains the appropriate volumes of all reagents required for multiple PCR reactions, saves time and greatly reduces tube-to-tube variation.

If multiple templates are being tested with the same primers, include the primers in the Master Mix. If one template is being tested with multiple primer sets, include the template in the Master Mix.

If you are setting up several sets of parallel samples, assemble multiple Master Mixes (e.g., each with a different set of primers). The Master Mix should be thoroughly mixed before use (i.e., vortexed without bubbling).

Experimental Procedures

A. Control PCR Reactions

The following control PCR reactions should be performed in parallel with your experiments to ensure that PCR Kit is working properly.

1. Place all components on ice and allow thawing completely. Mix each component thoroughly before use and spin tube briefly to collect all the liquid in bottom of tube.

2. For **each** PCR reaction, transfer **25µl** from PCR EZ MASTER MIX to a clean microcentrifuge tube.

3. Add in the tube, your specific Forward Primer, Reverse Primer and DNA template of interest to a total of **50µl** reaction volume.

4. [Optional]: For positive and negative control reaction tubes, please see below.

5. [Optional]: If your thermal cycler does not have a "hot lid", add 1-2 drops of mineral oil to prevent evaporation during cycling. A good "seal" of mineral oil should have a well-defined meniscus between the two phases. Cap the PCR tubes firmly.

6. Commence thermal cycling using the following parameters:

   –95°C for 5minutes
   –30 cycles
   –95°C for 50 seconds; 65°C for 50 seconds;
   72°C for 3 minutes
   –30 cycles with a 3-minutes annealing/extension time is sufficient for amplification of the positive control template provided in the kit. Other templates may require more or less cycles and different annealing/extension times.

B. Multiple products

Too many cycles
Reducing the cycle number may eliminate nonspecific bands.

Annealing temperature
Increase the annealing/extension temperature in increments of 2–3°C.

Suboptimal primer
Redesign your primer(s) after confirming the design accuracy of the sequence information. If the original primer(s) was less than 22 nt long, try using a longer primer. If the original primer(s) had a G-C content of less than 45%, try to design a primer with a G-C content of 45–60%.

C. Low yield

Poor template
Check template integrity by electrophoresis on a quality standard TBE-agarose gel. If necessary, re-purify your template using methods that minimize shearing and nicking.

D. Products are smeared on gel

Too many cycles
Reduce the cycle number by 3–5 to see if non-specific bands disappear.

Poor template
Check template integrity by electrophoresis on a quality denaturing agarose gel. Re-purify your template if necessary.

Troubleshooting Guide

The following general guidelines apply to most PCR reactions. However, no attempt has been made to address troubleshooting for all of the many applications.

A. No product observed

PCR component
Use a checklist when assembling reactions. If the positive control does not work, repeat the positive control only. If the positive control still does not work, repeat again replacing individual components to identify the faulty reagent.

Annealing temperature
Decrease the annealing temperature in increments of 2–3°C.

Poor template
Check template integrity by electrophoresis on a quality standard TBE agarose gel. If necessary, re-purify your template using methods that minimize shearing and nicking.

Extension time
Especially for longer templates, increase the short extension time in 1 minute increments.

Difficult target
Some targets are inherently difficult to amplify. In most cases, this is due to unusually high G-C content and/or secondary structure. Use special kit for GC rich template.

Reagent	Final Concentration	Quantity
PCR EZ MASTER MIX	---	**25μl**
Primer I, forward	0.1-1μM	variable
Primer II, reverse	0.1-1μM	variable
Template DNA	See note 1	variable
Water (PCR Grade)		
Total Volume	**---**	**50μl**

Reagent	Positive Control	Negative Control
PCR EZ MASTER MIX	**25μl**	**25μl**
Control Primer I(10μM), forward	1μl	1μl
Control Primer II(10μM), reverse	1μl	1μl
Control Lamda DNA Template, 10ng/ul	1μl	---
Water (PCR Grade)	22μl	23μl
Total Volume	**50μl**	**50μl**

7. Transfer 5 μl sample of your PCR reaction to a fresh tube and add 1 μl of 5X stop/loading buffer. Analyze your sample(s), along with suitable DNA size markers, by electrophoresis on a 1.2% agarose/EtBr gel.

   Expected results: If you are using the positive control reagents provided in the kit, the reaction should produce a single major fragment of 3 kb.

Notes for the Components of the Reaction Mixture:

1. **Template DNA:** Usually the amount of template DNA is in the range of 0.01-1ng plasmid or phage DNA and 0.1-1µg for genomic DNA, for a total reaction mixture of 50µl.

2. **Primers:** The PCR primers are usually 15-30 nucleotides in length, longer primers provide higher specificity. The GC content of primer should be 40-60%. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, and the melting temperature of flanking primers should not differ by more than 5°C. If the primer is shorter than 25 nucleotides, the approximate melting temperature is calculated using the formula as: **Tm=4(G+C) + 2(A+T)**.

3. **$MgSO_4$ concentration:** Since Mg^{2}+ ions form complex with dNTPs, primers and DNA templates, the optimal concentration of $MgSO_4$ has to be selected for each experiment. In our experiments, at a final dNTP concentration of 200µM, 2mM $MgSO_4$ concentration is suitable in most case.

4. **dNTPs:** The final concentration of each dNTP in the reaction mixture is usually 200µM.

5. **Taq DNA polymerase:** Usually 1-1.5U of Taq DNA polymerase is used in the 50µl of reaction mix. Higher Taq DNA polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of Taq DNA polymerase (2-3U) may be necessary to obtain a better yield of amplification products.

6. **Cycling conditions**: Usually denaturation for 0.5-2 minutes at 94-95°C is sufficient; the optimal annealing temperature is 5°C lower than the melting temperature of primer-template DNA duplex. Usually the extending step is performed at 70-75°C. Recommended extending time is 1 minute for the synthesis of PCR fragments up to 2kb. When larger DNA fragments are amplified, the extending time is usually increased by 1 minute for each 1kb.

7. **Number of cycles**: The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. For less than 10 copies of template DNA, 40 cycles should be performed. If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.

8. **Final extending step:** After the last cycle, the samples are usually incubated at 72°C for 5-15 minutes to fill-in the protruding ends of newly synthesized PCR products. Also, during this step, the terminal transferase activity of Taq DNA polymerase adds extra A nucleotides to the 3'-ends of PCR products.