

# BIOTAQ™ PCR Kit

Shipping: On Dry/Blue Ice.

Catalog numbers

Batch No.: See vial

BIO-21071: 500 Units



A Meridian Life Science® Company

Store at -20°C

## Storage and stability:

BIOTAQ™ PCR Kit is shipped on Dry/Blue Ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. When stored under optimum conditions, the reagents are stable for a minimum of 12 months from date of purchase.

## Unit Definition:

One unit is defined as the amount of enzyme that incorporates 10nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

## Storage and Dilution Buffer:

20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% glycerol and stabilizers.

## Associated Activities:

Endonuclease and exonuclease activities were not detectable after 2 and 1-hour incubation, respectively, of 1µg Lambda DNA and 0.22 µg of EcoR I digested Lambda DNA, respectively at 72°C in the presence of 15-20 units of BIOTAQ™ DNA polymerase.

## Note

1. Research Use Only.

## Features

- Ideal for setting up new procedures
- Designed for easy optimization
- Contains Bioline's ultra-pure dNTPs
- Supplied with 2x PolyMate Additive for difficult or "dirty" templates

## Applications

- Routine PCR applications
- TA cloning

## Description

The BIOTAQ™ PCR Kit contains all the necessary components to perform PCR assays on a wide range of DNA templates. In addition to dNTPs, the PCR Kit is based on our widely used BIOTAQ DNA Polymerase, which achieves dependable results.

BIOTAQ DNA Polymerase is a highly purified thermostable DNA polymerase offering very high yield over a wide range of PCR templates, and is the ideal choice for most assays. BIOTAQ is a robust preparation and consistently delivers high yields with minimal background. BIOTAQ possesses 5'-3' exonuclease activity and leaves an 'A' overhang such that the primer extension product is suitable for effective integration into TA cloning vectors.

## Components:

Reagent	Size
BIOTAQ DNA Polymerase	500 Units
10x NH <sub>4</sub> Reaction Buffer	2 x 1.2ml
50mM MgCl <sub>2</sub> Solution	1.2ml
10mM dNTP Mix	1ml
2x PolyMate Additive	1.2ml

## General Considerations:

This protocol serves as a guideline only. Reaction parameters such as Mg<sup>2+</sup> concentration, incubation times and temperatures will vary depending upon the system used and should be independently optimized for each set of reactions.

The optimal Mg<sup>2+</sup> concentration should be determined empirically, but in most cases 1.5mM will give satisfactory results.

Disposable pipette tips with hydrophobic filters should be used to minimize risk of cross-contamination.

All solutions should be thawed and then kept on ice. Repeated freeze thawing of buffers is not recommended, so appropriate aliquots should be made upon initial thawing. All solutions should be mixed well before use.

Specificity and performance can be improved by using 2 x Polymate Additive (provided). Compose the reaction mix, containing buffer, dNTPs, Mg<sup>2+</sup>, template DNA, primers, DNA polymerase. Add 2x PolyMate at the volume of half of the final volume of reaction (e.g. 25µl per 50µl final volume, etc). Add ddH<sub>2</sub>O up to final volume and mix by pipetting.

10mM Mix contains 2.5mM of each dNTP (supplied)

Reaction Volume	10mM dNTP Mix
50µl	5µl

For direct use in DNA synthesis *in vitro*. Add the 10mM dNTP Mix directly into the reaction mixture. We recommend a final concentration of between 1-2mM.

### PCR Reaction Conditions (for a 50µl reaction)

10x NH <sub>4</sub> Reaction Buffer	5µl
50mM MgCl <sub>2</sub> Solution	1.5 - 4.0µl
100mM dNTP Mix (see below)	5.0µl
Template and primers	As required
BIOTAQ	0.5 - 1µl
Water (ddH <sub>2</sub> O)	Up to 50µl

Denature: 94-96°C;

Extension: 70-72°C allowing 30-45 seconds per Kb

## Troubleshooting Guide

Problem	Recommendation
No or low PCR yield	For Difficult templates (AT and GC rich). Try 2x PolyMate (BIO-37041) to lower the melting profile and improve performance.
	Enzyme concentration too low – increase the amount enzyme in 0.5U increments.
	Magnesium concentration too low – increase concentration in 0.25mM increments.
	Primer concentration not optimised. Titrate primer concentration (0.3-1µM); ensuring that both primers have the same concentration.
Multiple bands	Primer annealing temperature too low. Increase annealing temperature. Primer annealing should be at least 5°C below the calculated Tm of primers.
	Prepare master mixes on ice or perform a hot-start step.
	For problems with low specificity. Try 2x PolyMate (supplied) to improve specificity.
Smearing or artifacts	Template concentration too high. Prepare serial dilutions of template.
	Too many cycles. Reduce the cycle number by 3-5 to remove non-specific bands.
	Enzyme concentration too high – decrease the amount of enzyme in 0.5U increments.
	Extension time too long. Reduce extension time in 0.5-1 minute increments.

## Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact your local distributor or our Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: [tech@bioline.com](mailto:tech@bioline.com)

## Citations: ([http://www.bioline.com/h\\_scholar.asp](http://www.bioline.com/h_scholar.asp))

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3. Bower, N.I. & Johnston, I.A. *Physiol. Genomics* **42a(2)**, 114-130 (2010).
4. Komatsu, M. *et al. J. Gen. Plant Pathol.* **76(6)**, 363-369 (2010).
5. Bower, N.I. *et al. J. Exp Biol.* **211**, 3859-3870 (2008).
6. Lopez-Lluch, G., *et al. PNAS* **103(6)**, 1768-1773 (2006).
7. Cervero, A., *et al. Clin. Endocrinol. Metab.* **89**, 2442–2451 (2004).
8. Brigido, C., *et al. Vet. Parasitol.* **123(1-2)**, 17-23 (2004).
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## Associated Products:

Product	Pack size	Cat. No.
Agarose, Molecular Grade	500g	BIO-41025
dNTP Mix	500µl	BIO-39028
2x PolyMate Additive	2 x 1.2ml	BIO-37041
HyperLadder 1kb	200 Lanes	BIO-33025

## Trademark and Licensing Information

- 1). Notice to Purchaser: Licensed under U.S. patent numbers 5,338,671 and 5,587,287 and corresponding patents in other countries
- 2). BIOTAQ is a Trademarks of Bioline.Ltd

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