Product Insert

BIOTAQ™ PCR Kit

Catalogue Number:
BIO-21071 500 Units

Features

- Ideal for setting up new procedures
- Designed for easy optimizations of PCR applications
- Contains ultra-pure dNTPs manufactured by Bioline
- Supplied with 2x PolyMate Additive for difficult or "dirty" templates

Applications

- Routine PCR applications
- Products suitable for 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.

Kit Components

BIOTAQ DNA Polymerase	Size
BIOTAQ™ DNA polymerase @ 5u/µl	500 Units
10x NH_4 Buffer	2 x 1.2ml
50mM $MgCl_2$ Solution	1.2ml
10mM dNTP Mix	1ml
2x PolyMate Additive	1.2ml

Reagent Specifications:
10x NH_4 Reaction Buffer: 160mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% stabilizer
$MgCl_2$ Stock Solution: 50mM $MgCl_2$ (suggested final concentration 1.5mM – 4mM).

10mM dNTP Mix: Ultra-pure dNTPs are manufactured by Bioline and consist of a mix of
dATP, dGTP, dCTP, and dTTP as Lithium salts (pH 7.5).

2x PolyMate Additive: Provides an optimized composition of reagents, and is ideally suited to dirty/difficult templates with GC or AT-rich DNA, repetitive sequences or sequences with a high level of secondary structure. PolyMate acts as a melting agent which enables the DNA polymerase and oligonucleotides to provide greater access to the template DNA.

Product Specifications

Batch details:
Batch No: See vial
Units per vial: See vial
Concentration: See vial

Storage Buffer:
20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% Glycerol, and stabilizers.

Storage Conditions:
BIOTAQ PCR Kit can be stored for 12 months at -20°C.

Shipping Conditions:
On Dry Ice or Blue Ice.

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.

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

Associated Products:

Product Name	Pack Size	Cat No
Hyper Ladder I	200 Lanes	BIO-33025
HyperLadder IV	200 Lanes	BIO-33029
Agarose	100g	BIO-41026

Product Citations:

1. Melo, M., & Hansson, B. *Molecular Ecology Notes* **6(4)**, 1266 (2006).
2. Robba, L., *et al*. *American Journal of Botany* **93,**1101-1108 (2006).
3. Rens, W., *et al*. *Nature Protocols* **1,** 783-790 (2006).

Notes
1. BIOTAQ is a Trademark of Bioline.
2. This product insert is a declaration of analysis at the time of manufacture.
3. Research Use Only

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See Overleaf for Reaction Conditions and Recommendations

Reaction Conditions (for a 50µl PCR reaction):

10x NH_4 Buffer	5µl
$MgCl_2$ 50mM Solution	1.5-4µl
10mM dNTP Mix	5µl
Template and primers	as required
BIOTAQ™ @ 5u/µl	0.5-1µl

Denaturation: 94-96^{o}C
Elongation: 70-72^{o}C (allowing 30-45 sec./Kb)

General Considerations:

- This protocol serves as a guideline only. Reaction parameters such as Mg^{2+} concentration, incubation times and temperatures will vary depending upon the system used and should be independently optimised for each set of reactions.
- The optimal Mg^{2+} 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 minimise 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^{2+}, template DNA, primers, DNA polymerase. Add 2x PolyMate at the volume of half of the final volume of reaction (e.g. 25ul per 50ul final volume, etc). Add ddH_2O up to final volume and mix by pipetting.
- 10mM dNTP 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 Troubleshooting Guide

Observation	Recommended solution(s)
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^{o}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 artefacts	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.