Product Insert

BIOTAQ™ Core Kit

Research Use Only

Catalogue No: BIO-21071 500u

Description:
The BIOTAQ™ Core kit contains all reagents necessary for labs to carry out assays on most DNA templates. The user needs only to provide template and primers in accordance with the desired reaction.

Kit Contents	**Size**
BIOTAQ™ DNA Polymerase	500u
10x NH_4 Buffer	2 x 1.2ml
$MgCl_2$ (50mM) Solution	1.2ml
40mM dNTP Mix	1ml
2x Poly-Mate additive	1.2ml

BIOTAQ™ is a thermostable DNA polymerase purified from *Thermus aquaticus* (1). BIOTAQ™ offers consistent results across a wide range of assays. BIOTAQ™ leaves an A' overhang such that the primer extension product is suitable for effective integration into TA cloning vectors.

10x NH_4 Buffer (Mg^{2+} free) has been specially developed for BIOTAQ™ to give optimal conditions.

50mM $MgCl_2$ solution is supplied for individual reaction optimization.

A **40mM dNTP** Mix is also supplied; Bioline Ultrapure dNTPs are a mix of dATP, dGTP, dCTP, and dTTP lithium salts (pH 7.0).

A **2x Poly-Mate additive** is included for increased performance and specificity of difficult targets. Poly-Mate acts as a "melting" agent for dirty or difficult templates with high GC-, or AT- content, repetitive sequences or templates with difficult melting profiles. Poly-Mate optimizes primer extension by making DNA more easily accessible to Polymerase and oligonucleotides.

Storage Conditions:
BIOTAQ and dNTP mix can be stored at -20°C, in a constant temperature freezer for 12 months. BIOTAQ™ will remain stable if stored as specified.

Repeated freeze-thaw cycles will affect the stability of NH_4 Buffer and Poly-Mate Additive. These components will remain stable at +4°C for a minimum of one month.

Shipping Conditions
At +4ºC or -20ºC

Batch details:

Batch No: See vial

Units per vial: See vial
Concentration: See vial

Protocol
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 optimized for each set of reactions.

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.

The optimal Mg^{2+} concentration should be determined empirically, but in most cases 1.5mM will give satisfactory results.

The following components should be combined in a thin-walled PCR tube:

For a 50µl Reaction:	
10x NH_4 Buffer	5µl
$MgCl_2$ 50mM Solution	1.5-4µl
40mM dNTP Mix	1.25µl
Template and primers	as required
BIOTAQ (5u/µl)	2.5µl
Water (ddH_2O)	upto 50µl

Guideline parameters:

Denaturation 94-96°C
Elongation 70-72°C (allowing 30-45 sec./kb)

2x Poly-Mate Additive
Compose the reaction mix, containing buffer, dNTPs, Mg^{2+}, template DNA, primers, DNA polymerase. Add 2x Poly-Mate at the volume of half of the final volume of reaction (e.g. 25ul per 50ul final volume, etc). Add ddH2O up to final volume and mix by pipetting.

40mM Mix contains 10mM of each dNTP

Reaction Volume	Master Mix
50µl	1.25µl

For direct use in DNA synthesis *in vitro*. Add the 40mM dNTP Mix directly into the reaction mixture. We recommend a final concentration of between 1-2mM.

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.

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

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

Reaction buffer:
NH_4 buffer (10x): 160mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20.

Mg^{2+} Stock solution:
50mM $MgCl_2$ (suggested final concentration 1.5mM-4mM).

References: (1) Kaledin, A.S., Slyuisarenko, A.G. and Gorodetskii, S.I. (1981) Biokhimiya **46**, 1576

This product contains a declaration of analysis at the time of manufacture