

Certificate of Analysis

Taq-Pro Core Kit

Research Use Only

Catalogue No: CB-4075-1 500u

Description:

The TAQ-Pro 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
Taq-Pro DNA Polymerase	500u
10x NH ₄ Buffer	2 x 1.2ml
MgCl ₂ (50mM) Solution	1.2ml
40mM dNTP Mix	1ml
2x Clear-Band Solution	1.2ml

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

10x NH₄ Buffer (Mg²⁺ free) has been specially developed for BIOLASE to give optimal conditions.

50mM MgCl₂ solution is supplied for individual reaction optimization.

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

A **2x Clear-Band solution** is included as an enhancer and specificity of difficult targets. Clear-Band Solution acts as a "melting" agent for dirty or difficult templates with high GC-, or AT- content, repetitive sequences or templates with difficult melting profiles. Clear-Band Solution renders DNA more accessible to Polymerase and oligonucleotides.

2x Clear-Band Solution

Compose the reaction mix, containing buffer, dNTPs, Mg²⁺, template DNA, primers, DNA polymerase. Add 2x Clear-Band 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.

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 Mg²⁺ concentration, incubation times and temperatures will vary depending upon the system used and should be independently optimised 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 Mg²⁺ 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
Taq-Pro (5u/µl)	2.5µl
Water (ddH ₂ O)	upto 50µl

Guideline parameters:

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

2x Clear-Band Additive

Compose the reaction mix, containing buffer, dNTPs, Mg²⁺, template DNA, primers, DNA polymerase. Add 2x Clear-Band 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 1 µg 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:

NH₄ buffer (10x): 160mM (NH₄)₂SO₄, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20.

Mg²⁺ Stock solution:

50mM MgCl₂ (suggested final concentration 1.5mM-4mM).

Storage Conditions:

The Taq-Pro Core Kit can be stored at -20°C, in a constant temperature freezer for 12 months. The Taq-Pro Core Kit will remain stable if stored as specified.

Shipping Conditions:

At +4°C or -20°C.

Repeated freeze-thaw cycles will affect the stability of NH₄ Buffer and Clear Band Additive. These components will remain stable at +4°C for a minimum of one month.

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

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This certificate is a declaration of analysis at the time of manufacture