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3B ULTRATOOLS DNA POLYMERASE 1 U/µl

PRODUCT	FORMAT	REF.
UltraTools DNA Polymerase 1U/µl-Standard reaction Buffer	100U	3B064
UltraTools DNA Polymerase 1U/µl-Standard reaction Buffer	250U	3B065
UltraTools DNA Polymerase 1U/µl-Reaction Buffer $MgCl_2$ Free	100U	3B068
UltraTools DNA Polymerase 1U/µl- Reaction Buffer $MgCl_2$ Free	250U	3B069

1. GENERAL CONSIDERATIONS

Highly thermostable DNA polymerase. It is a recombinant, modified form of the enzyme from the thermophilic bacterium *Thermus thermophilus* expressed in *E. coli.*

3B BlackBio Biotech ULTRATOOLS DNA polymerase is suitable for applications which require a highly thermostable and processive enzyme capable of synthesising DNA strands at elevated temperatures in DNA amplification reactions or similar (e.g. primer extension), thus resolving the most complex secondary structures.

Our ULTRATOOLS DNA polymerase is also recommended for non-stringent applications (e.g. **RAPDs**). It is the enzyme of choice for applications involving **bacterial DNA sequences homologous to those found in *E. coli*.**

The enzyme is free of unspecific endo- or exonucleases activities, as well as nicking activities. It does not either exhibit significant reverse-transcriptase activity. Terminal transferase activity inherent to the enzyme renders A-tailed amplification products suitable to be further used in T/A cloning approaches

*The enzyme is supplied at a **concentration of 1 U/µl** in a storage buffer. This concentration allows accurate pipetting of small amounts of the DNA polymerase, so that it is not necessary to perform ulterior dilutions.*

Unit Definition

One unit is defined as the amount of enzyme which incorporates 10 nanomoles of dNTPs into acid-insoluble DNA within 30 minutes at 72 ºC.

Reaction buffer

Recommended reaction buffer is: 75 mM Tris HCl (pH 9.0), **2 mM $MgCl_2$** (see Note 1), 50 mM KCl, 20 mM $(NH_4)_2SO_4$. This reaction buffer (the so-called Standard Buffer, Ordering Information at the end) is supplied at 10X concentration together with the enzyme (either accompanying it, or included in it, in the case of aliquoted vials).

Reaction buffer can be supplied $MgCl_2$ free (the so-called Free Buffer, see Ordering Information): Mg^{2+} ion, being the enzyme cofactor, plays a key role on polymerase activity, this is why its concentration must be optimised in certain amplification-based experiments. In this case, the $MgCl_2$ is supplied as a separate vial at 50 mM concentration. This solution must be completely thawed, vigorously vortexed and spun down in a bench-top centrifuge before use.

Storage Conditions

Store at -20ºC in a **constant temperature freezer** (i.e. do not use frost-free freezers)**.** Under these conditions the activity of the enzyme remains unaltered over 18 months of storage. The glycerol in the storage buffer prevents freezing at -20ºC.

Reaction Conditions

After thawing the reaction buffer (and $MgCl_2$ solution, in case the "free buffer" choice is adopted), shake all vials (buffer, enzyme, 50 mM $MgCl_2$ solution) by gentle vortexing, later spin them down in a bench-top centrifuge, and eventually pipette desired volumes.
Keep all reagents on ice while they remain out of the -20ºC storage freezer, otherwise enzyme activity will decrease over the time. Wear disposable gloves and make use of sterile, DNase- and RNase-free pipette tips and tubes in order to avoid contaminations and false negative results.

Recommended enzyme volumes to be added to the reaction mix

Final reaction volumes	Recommended enzyme volumes
100 µl	Up to 2.5 µl
50 µl	1-1.25 µl
25 µl	0.5-0.75 µl

It is recommended to increase the enzyme units (up to three times) in order to perform certain applications such as PRINS (Primed In Situ Synthesis) or when working on long DNA fragment amplifications (longer than 2 Kb from genomic DNA).

The dNTP final concentration recommended is 200 µM ,but this figure may be altered (e.g. when unspecific amplimers occur), enlarged (e.g. long amplifications) or even unbalanced in favour of any dNTP in particular (e.g. in vitro mutagenesis experiments) depending on the intended approach. This enzyme also accepts modified dNTPs (e.g. radioactively or fluorescein labelled) as substrate.

Notes

Note 1: at difference with the vast majority of the thermostable DNA polymerases existing in the market,our ULTRATOOLS DNA polymerase shows optimal specificity at 2 mM $MgCl_2$ final concentration (rather than 1.5 mM) in reaction buffer.