Product Insert

Diamond DNA Polymerase

Research Use Only

Product:
Diamond DNA Polymerase

Description:
Diamond DNA Polymerase is designed to cope with difficult templates, such as high GC% regions and microsatellites, and is the polymerase of choice for applications where specificity / low background is crucial.

Catalogue No:
BIO-21058 250u
BIO-21059 500u

Batch details:

Batch No:	See vial
Units per vial:	See vial
Concentration:	5 u/µl

Additional reagents supplied:
10x NH_4 Reaction Buffer: 160mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20.
$MgCl_2$ Stock Solution: 50mM $MgCl_2$

Features:
- Extremely high specificity
- Highly suited to GC% rich templates.
- The ability to polymerise through regions of DNA such as secondary structures or micro-satellites, which are difficult to extend with other polymerases.
- Elimination of artifacts caused by non-specificity.
- Maintains specificity in conditions designed for high performance (high Mg^{2+}, dNTPs and primer concentrations), almost eliminating by-product formation.

Suggestions for use:

Reaction Conditions (for a 50µl reaction)

10x NH_4 Buffer	5µl
50mM $MgCl_2$ Solution	2-10µl
100mM dNTP Mix (see below)	0.5-1.0µl
Template and primers	as required
Enzyme	0.2-1.0µl
Water (ddH_2O)	up to 50µl

Bioline 100mM dNTP Mix is available as a separate product (Catalogue number BIO-39028)

Denature: 94-97°C
Extension: 72°C Allowing 2 mins per Kb

This data is intended for use as a guide only, conditions will vary from reaction to reaction and may need optimisation.

It is advisable to determine the optimum concentration of Magnesium. Start with titrations of 2 – 7 mM (final conc.). With certain templates, ±0.5mM Mg^{2+} can make a huge difference. For <500 bp size, the use of 2.5 u of enzyme in a 50µl reaction is recommended, whereas 5 u is recommended for 500-1500 bp size. *(For initial tests, it is advisable to run a positive control reaction using BIOTAQ DNA polymerase under standard conditions.)*

Owing to the extremely high specificity shown by Diamond, it is possible to use $MgCl_2$ concentrations higher than 10mM, i.e. Diamond may be used for applications requiring very high Mg levels. It is recommended to allow 1 to 2 minutes of DNA extension per 1kb.

Applications:
When templates are challenging, such as high GC% or repeat sequences, the conditions required by traditional polymerases lead to poor specificity. Diamond addresses this problem since the enzyme maintains excellent specificity and minimal background. In fact, even on genomic templates, Diamond can be used with $MgCl_2$ concentrations as high as 10mM. Furthermore, Diamond is capable of extending through regions that are difficult with other polymerases, such as inverted tandem repeats and those with high amounts of secondary structure. Diamond works in a totally unique way, involving improved nucleotide selection and a lower rate of mis-match extension, meaning that only perfectly aligned primers will be extended. However, because Diamond is so specific, it is necessary to determine carefully the optimum conditions (Mg^{2+} concentration, annealing temp), for use with each template/primer set. Diamond has a very weak terminal transferase activity, and products can be assumed to be blunt-ended. However, this is sequence-dependent, and some sequences may be tailed with a single nucleotide.

Specificity and Performance of Diamond DNA Polymerase can be increased with the use of **2x Poly-Mate** (not supplied), which is designed for GC- or AT-rich DNA, "dirty" templates or sequences with difficult melting profile.

Storage Conditions:
Diamond DNA Polymerase can be stored at -20°C, in a constant temperature freezer for 12 months. Diamond will remain stable if stored as specified.
Storage and dilution buffer:
20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% Glycerol, and 0.1% Tween-20.

Shipping Conditions:
At +4ºC or -20ºC.

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.

Associated activities
Endonuclease and exonuclease activities were not detectable after 4 hours incubation of 1µg of pBR322 plasmid DNA and 0.5 µg of *Hind III*-digested lambda DNA at 72°C in the presence of 20 units of Diamond DNA polymerase.

Associated products

Product Name	Pack Size	Cat No
dNTP Set	4 x 25µmol	BIO-39025
dNTP Mix 100 mM total	1 x 500 µl	BIO-39028
dNTP Mix 40 mM total	1 x 500 µl	BIO-39043
2x Poly-Mate Additive	2 x 1.2ml	BIO-37041
Bio-X-Act Short DNA polymerase	250 units	BIO-21064
Bio-X-Act Short DNA polymerase	500 units	BIO-21065
Hyper Ladder I	200 lanes	BIO-33025
Hyper Ladder I	500 lanes	BIO-33026
Agarose	500g	BIO-41025

Note: This product is supplied for use in primer extension reactions.
Purchase of this product does not convey a licence to perform any patented process.

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