Product Insert

VELOCITY DNA Polymerase

Catalogue Numbers:
BIO-21098 250 Units
BIO-21099 500 Units

Features

- High-speed, high fidelity DNA polymerase
- High processivity
- Fast amplification
- Shorter PCR runs
- Robust, requires minimal optimization of the reaction

Applications

- Cloning techniques where high fidelity is desirable
- Blunt-end cloning
- Amplification of difficult templates
- Mutagenesis

Description

VELOCITY is a fast thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proofreading exonuclease activities. VELOCITY provides high fidelity with an error-rate of 8 x 10^{-7} combined with a high speed DNA synthesis as fast as 15s/Kb for templates of up to 5Kb and 30s/Kb for templates longer than 5Kb. The inherent high speed DNA synthesis is due to the enhanced processivity of the polymerase.

VELOCITY is easy to use since it works with many different protocols and requires minimal optimization. The polymerase produces higher yields than most commercially available enzymes and generates blunt-ended amplicons. The error rate of VELOCITY DNA Polymerase (8 x 10^{-7}) was determined using the rpsL fidelity assay (Lackovich et al., 2001; Fujii et al., 1999).

Two buffers are provided with VELOCITY DNA Polymerase. For general high fidelity applications, use 5x Hi Fi Reaction Buffer. For GC-Rich or difficult templates, use 5x GC Reaction Buffer.

Product Specifications

Batch details:

Batch No:	See vial
Units per vial:	See vial
Concentration:	2u/µl

Components

	250 Units	500 Units
VELOCITY DNA Polymerase	125µl	250µl
5x HI-Fi Buffer (contains 10mM Mg^{2+})	2 x 1.5ml	4 x 1.5ml
5x GC Buffer (contains 10mM Mg^{2+})	2 x 1.5ml	4 x 1.5ml
50mM $MgCl_2$ Solution	1.2ml	1 x 1.2ml

VELOCITY Storage Buffer: 10mM Tris-HCl, pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT, glycerol and stabilizers.

Storage Conditions:
VELOCITY DNA Polymerase can be stored for 12 months at -20°C.

Shipping Conditions:
On Dry Ice or Blue Ice

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 Products:

Product Name	Pack Size	Cat No
dNTP Set	4 x 25µmol	BIO-39025
dNTP Mix	500µl	BIO-39028
HyperLadder I	200 Lanes	BIO-33025
Agarose Tablets	150g	BIO-41028
5x Hi-Specc Additive	3 x 1.2ml	BIO-37032

Reaction Conditions (for a 50µl volume)

5x Hi-Fi or 5x GC Reaction Buffer	10µl
100mM dNTP Mix (see below)	0.5µl*
Template and primers	as required*
Enzyme	0.5–2 Units*
Water (ddH_2O)	up to 50µl

Owing to VELOCITY's inherent 3'-5' exonuclease activity, the enzyme must be added last to a reaction in order to prevent primer degradation.

Bioline 100mM dNTP Mix is available as a separate product (see associated products).

Cycling Conditions:
Initial denaturation 96-98°C for 0.5-2min

Amplification:
Denaturation 96-98°C 30s
Annealing X°C 30s X ~5°C of the lower Tm primers
Extension 72°C

Use extension time of 15s per 1kb for plasmid template up to 5kb.
Use extension time of 30s per 1kb or plasmid template >5kb and genomic DNA

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

***General Considerations:**
The suggested final concentration of Mg^{2+} in the reaction is likely to be 2-4mM, but some optimisation may be necessary to achieve the best possible results. Please, note that the reaction buffer already provides 2mM Mg^{2+}(final concentration).

Enzyme:
We recommend a range of 0.5–2.0 Units of VELOCITY in a 50µl reaction. Do not exceed 2u/50µl.

Concentration dNTP:
We recommend 250µM (final conc.) of each dNTP. Do not use dUTP.

Buffers:
For general high fidelity applications, use 5x Hi-Fi Buffer. For GC-rich or difficult templates, use 5x GC-Buffer. For further improvement of results, Hi-Spec Additive (not supplied) can be used to eliminate unwanted by-products such as background smears and spurious bands. Hi-Spec Additive (Cat. No.BIO-37032)

Primers:
Use 10-100pmol of each primer per 50µl reaction volume.
We recommended 20pmol per 50µl reaction volume.

Template:
Use approximately 50pg-10ng per 50µl reaction volume for plasmid template up to 5kb.
Use approximately 500pg-25ng per 50µl reaction volume for genomic DNA and plasmid template >5kb.

1. VELOCITY 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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