VELOCITY DNA Polymerase

Shipping: On Dry/Blue Ice

Batch No.: See vial

Concentration: 2 U/µL

Catalog numbers

BIO-21098: 250 Units

BIO-21099: 500 Units

Store at –20 °C

meridian BIOSCIENCE™

Storage and stability:
The VELOCITY is shipped on dry/blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thaw, mix, and briefly centrifuge each component before use.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Please refer to the material safety data sheet for further information.

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.

Quality control specifications:
VELOCITY and its components are extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

Notes:
For research or further manufacturing use only.

Description

VELOCITY is a fast, proofreading DNA polymerase from archaeal origin which generates blunt-ended amplicons. Its high thermostability combined with its 5'-3' DNA polymerase and 3'-5' proofreading exonuclease activities makes VELOCITY an ideal enzyme for all PCR applications. Indeed, VELOCITY possesses an error-rate of 4.4 x 10^{-7}, providing a 50-fold higher fidelity than *Thermus aquaticus* DNA polymerase (determined using an adapted rpsL fidelity assay, Mo J.Y. *et al., J. Mol. Biol.* 1991; Fujii. S. *et al., J. Mol. Biol.* 1999). In addition, owing to its enhanced processivity, VELOCITY exhibits not only high amplification rates up to 66 bp/s (equivalent to 15 s/kb), but also results in higher yields than most commercially available enzymes.

Components

Product Name	250 Units	500 Units
VELOCITY DNA Polymerase	125 µL	250 µL
5x Hi-Fi Buffer (contains 10 mM Mg^{2+})	2 x 1.5 mL	4 x 1.5 mL
50 mM $MgCl_2$ Solution	1 x 1.2 mL	1 x 1.2 mL
DMSO	1 x 1.25 mL	1 x 1.25 mL

General Considerations and Optimization

The optimal conditions will vary from reaction to reaction and are dependent on the system used. Each parameter has to be adjusted individually and some optimization may be required.

dNTP:
For optimal results we recommend using ultra pure dNTPs in a balanced mix. We recommend a final concentration of 250 µM each. Do not use dUTP or dITP

Mg^{2+}:
The optimal Mg^{2+} concentration depends on the dNTP concentration used. Since a 1:2 ratio (dNTP:Mg^{2+}) is usually optimal, we recommend a final Mg^{2+} concentration of 2 mM, but some optimization may be necessary, especially if using dNTP concentration higher than the recommended one. Indeed, a non-optimal concentration of Mg^{2+} leads to inefficient dNTP incorporation by the DNA polymerase. Since Mg^{2+} is also able to bind to DNA, an excess of Mg^{2+} in the reaction will promote secondary structure elements and increase non-specific primer binding leading to non-specific products. Alternatively, too low a concentration will decrease the reaction yield.

Please note that the reaction buffer already provides 2 mM Mg^{2+} (final concentration).

Enzyme:
We recommend a range of 1.0–2.0 Units of VELOCITY in a 50 µL reaction. We suggest to start with the lowest concentration and not to exceed 2 u/50 µL.

Buffers and DMSO:
The default buffer is 5x Hi-Fi Buffer and has been designed to give high yield and fidelity for the majority of standard templates, however we would recommend the addition of 3% DMSO (final concentration) for optimal performance.

For difficult templates such as genomic DNA or those possessing high-GC -content or complex structural organisation, a higher concentration of DMSO could be advantageous. We would recommend doing a titration up to 10% DMSO, however in this case the annealing temperature should be reduced since DMSO decreases the melting point of primers by up to 5°C.

Primers:
Forward and reverse primers are generally used at the final concentration of 0.2-0.6 µM each. We recommend as a starting point, to use 0.4 µM final concentration (*i.e.* 20 pmol of each primer per 50 µL reaction volume). Too high primer concentration can reduce the specificity of the priming, resulting in non-specific products.

Template:
The amount of template in the reaction depends mainly on the type of DNA used. For templates with low secondary structural complexity such as plasmid DNA or λ Genomic DNA, we recommend using 50 pg-10 ng DNA per 50 µL reaction volume. For templates >5 kb and genomic DNA we recommend a starting amount of 200 ng DNA per 50 µL reaction, this can be varied between 5 ng-500 ng. Furthermore, it is important to avoid, where possible, using template re-suspended in EDTA-containing solution (*e.g.* TE buffer) since EDTA chelates free Mg^{2+}.

Initial denaturation: This step is important to completely denature the template in order to allow primers to bind specifically to the single-stranded DNA. We recommend performing the initial denaturation at 98 °C for 2 min. Although these conditions will be enough for most of the templates, it is possible to increase the initial denaturation time to up to 5 min for very complex templates such as chromatin.

Annealing temperature:
The annealing temperature depends upon the primers' sequences and is usually 2-5 °C below the lower Tm of the pair. We recommend starting with a 55 °C annealing temperature and if necessary to run a temperature gradient to determine the optimal annealing temperature.

Extension temperature and time:
The extension step should be performed at 72 °C. The extension time depends on the length and type of the product to be amplified. Owing to the high processivity of VELOCITY, an extension time of 15 s/kb can be used for low-complexity templates such as lambda genomic DNA or plasmid DNA. For templates with complex secondary structure such as human genomic DNA, we recommend using 30 s/kb.

Since the length of the amplicon is also an important parameter, we also recommend using 15 s/kb for amplicons <5 kb. For longer amplification, increase up to 1 min/kb the extension time.

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Standard Protocol

The following protocol given here is for a standard 50 µL reaction and can be used as a starting point for reaction optimization. The following reagents, once thawed, are kept on ice and mixed as described in a nuclease-free microcentrifuge tube.

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

PCR reaction setup:

5x Hi-Fi Reaction Buffer	10 µL
100 mM dNTP Mix	0.5 µL
Template	as required
Primers 20 µM each	1 µL
Enzyme	1 µL
DMSO (if required)	(1.5 µL)
Water (ddH_2O)	up to 50 µL

Standard cycling conditions:

Step	Temp.	Time	Repeat
Initial denaturation	98 °C	2 min	1
Denaturation	98 °C	30 s	25-35
Annealing	50-68 °C	30 s	
Extension	72 °C	15-30 s/kb	
Final extension (optional)	72 °C	4-10 min	1

Troubleshooting guide

Problem	Possible Cause	Recommendation
No PCR product	Missing component	- Check mix set-up and volumes used
	Defective component	- Check the aspect and the concentrations of all components as well as the storage conditions. If necessary test each component individually in control reactions
	Enzyme concentration too low	-Increase enzyme quantity to up to 2 U/50 µL reaction
	Cycling conditions not optimal	- Decrease the annealing temperature - Run a temperature gradient to determine the optimal annealing temperature - Increase the extension time, especially if amplifying long target - Increase the number of cycles
	Not enough Mg^{2+}	- Increase the $MgCl_2$ concentration in 0.5 mM increments
	Difficult template	- Increase the denaturation time - Add DMSO. We recommend starting with 3% final concentration and if necessary increasing it up to 10 %
Smearing or Non Specific products	Excessive cycling	- Decrease the number of cycles
	Extension time too long	- Decrease the extension time
	Annealing temperature too low	- Increase the annealing temperature - Titrate DMSO from 3 % to 10 % (final concentration)
	Too much enzyme	- Decrease enzyme concentration
	Primer concentration too high	- Decrease primer concentration
	Contamination	- Replace each components in order to find the possible source of contamination - Set-up the PCR reaction and analyze the PCR product in separated areas.

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