

VELOCITY™ PCR Kit

Shipping: On Dry/Blue Ice

Catalog numbers

Exp. Date: See vial

BIO-21104 : 20 reactions

Batch No.: See vial

Storage and stability: The VELOCITY PCR Kit is shipped on Dry/Blue Ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. When stored under optimum conditions, the reagents are stable for a minimum of 12 months from date of purchase.

Unit definition: One unit of VELOCITY is defined as the amount of enzyme that incorporates 10nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

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

Safety precautions: Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Notes:
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Store at -20°C

Description

The VELOCITY™ PCR Kit contains all necessary components for high-fidelity PCR and subsequent preparation of the PCR product for TA cloning.

VELOCITY PCR products are blunt ended due to the proofreading activity of the polymerase. Since 3'-A overhangs are necessary for TA cloning, the kit contains Bioline's PCR Tailing Mix, which adds a single adenine base whilst simultaneously inhibiting the proofreading activity of VELOCITY DNA Polymerase. Consequently, no purification steps are required, allowing direct TA cloning of the DNA using the newly synthesized 3'-A overhang.

Kit Components

Product Name	20 Reactions
VELOCITY DNA Polymerase (250 units)	125µl
5x Hi-Fi Buffer (contains 10mM Mg ²⁺)	2 x 1.5ml
50mM MgCl ₂ Solution	1 x 1.2ml
DMSO	1 x 1.25ml
10mM dNTP Mix	625µl
10x PCR Tailing Mix	100µl

The VELOCITY PCR kit contains enough VELOCITY DNA Polymerase, dNTPs, MgCl₂ and DMSO for optimizing your PCR reactions and enough PCR Tailing Mix for 20 tailing reactions (as optimization is not necessary).

Procedure

The VELOCITY PCR Kit follows a two step protocol; Step 1: high-fidelity PCR mediated by VELOCITY DNA Polymerase; Step 2: addition of a 3'-A overhang by the PCR Tailing Mix.

Step 1: High-fidelity PCR

The following protocol given here is for a standard 50µl 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.

5x Hi-Fi Reaction Buffer	10µl
10mM dNTP Mix	5µl
Template	as required
Primers 20µM each	1µl
VELOCITY DNA Polymerase	1µl
DMSO (if required)	(1.5 µl)
PCR Water (BIO-37980)	up to 50µl

PCR reaction setup:

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.

Standard cycling conditions:

Step	Temperature	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	15-30s /kb	
Final extension (optional)	72°C	4-10 min	1

Step 2: Addition of 3'-A overhang

3'-A tailing setup:

Step 1 PCR product	50µl
10x PCR Tailing Mix	5µl

Incubate at 72°C for 5 minutes.

Use 1µl of the reaction for direct TA cloning.

VELOCITY PCR 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 Bioline ultra pure dNTPs in a balanced mix. We recommend a final concentration of 250µM each. Do not use dUTP or dITP

Mg²⁺:

The optimal Mg²⁺ concentration depends on the dNTP concentration used. Since a 1:2 ratio (dNTP:Mg²⁺) is usually optimal, we recommend a final Mg²⁺ concentration of 2mM, but some optimization may be necessary, especially if using dNTP concentration higher than the recommended one. Indeed, a non-optimal concentration of Mg²⁺ leads to inefficient dNTP incorporation by the DNA polymerase. Since Mg²⁺ is also able to bind to DNA, an excess of Mg²⁺ 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 2mM Mg²⁺ (final concentration).

Enzyme:

We recommend a range of 0.25–2.0 Units of VELOCITY in a 50µl reaction. We suggest to start with the lowest concentration and not to exceed 2u/50µl.

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.

For difficult templates such as genomic DNA or those possessing high-GC-content or complex structural organisation, results may be enhanced by the addition of 1.5µl DMSO in 50µl reactions (3% final concentration). If needed, a higher concentration of up to 10% can be used. In this case the annealing temperature should be reduced since 10% 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 0.2-0.6µM each. We recommend as a starting point, to use 0.4µM final concentration (*i.e.* 20pmol of each primer per 50µl 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 50pg-10ng DNA per 50µl reaction volume. For templates >5kb and genomic DNA we recommend a starting amount of 200ng DNA per 50µl reaction, this can be varied between 5ng-500ng. Furthermore, it is important to avoid, where possible, using template re-suspended in EDTA-containing solution (*e.g.* TE buffer) since EDTA chelates free Mg²⁺.

Annealing temperature:

The annealing temperature depends upon the primers' sequences and is usually 2-5°C below the lower T_m 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 15s/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 30s/kb.

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

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 2U/50µl 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 0.5mM 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 conc.)
	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.

Notes

VELOCITY is a Trademark of Bioline.
This product insert is a declaration of analysis at the time of manufacture.
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