PRECISOR High-Fidelity DNA Polymerase PLUS

Cat. No. 1706-5-BL
Cat. No. 1706-125-BL

For research use only.
Store at -20°C.

PRECISOR High-Fidelity DNA Polymerase PLUS

1. Features

- High-speed, high-fidelity thermostable DNA polymerase
- High processivity - increased yields even from challenging templates
- Amplification of templates up to 30kb
- Shorter PCR runs for longer templates
- Robust - minimal optimization required

2. Applications

- Cloning techniques for which high fidelity is desirable
- Blunt-end cloning
- Amplification of difficult templates
- Site-directed mutagenesis

3. Description

PRECISOR High-Fidelity DNA Polymerase is a fast thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proofreading exonuclease activities. It provides exceptional fidelity combined with enhanced processivity resulting in high speed DNA synthesis (15s/kb for templates of up to 5kb and 30s/kb for templates longer than 5kb). PRECISOR High-Fidelity DNA Polymerase 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.

4. Contents

	1706-5-BL (5 rxns)	**1706-125-BL (125 rxns)**
PRECISOR High-Fidelity DNA Polymerase	25 Units (2U/µl)	250 Units (2U/µl)
5x HiFi Buffer (contains 10mM Mg^{2+})	400 µl	2 x 1.5 ml
10 mM dNTP Mix (dATP, dCTP, dGTP, dTTP; 2.5 mM each)	30 µl	625 µl
DMSO	1.25 ml	1.25 ml
50mM $MgCl_2$ Solution	200 µl	1.2 ml
5x GC Buffer (contains 10mM Mg^{2+})	400 µl	1.6 ml

Buffers and DMSO
5x HiFi Buffer, DMSO and 5x GC Buffer are provided with PRECISOR High-Fidelity DNA Polymerase.

- 5x HiFi Buffer for high-fidelity applications
- DMSO for difficult templates such as genomic DNA or those possessing complex structural organisation
- 5x GC Buffer for the amplification of GC-rich (GC content >65%) fragments.

For more details, please see the ‚General Considerations' section.

PRECISOR High-Fidelity DNA Polymerase Storage Buffer
10mM Tris-HCl pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT, glycerol and stabilizers.

Storage Conditions
All components should be stored at -20°C upon receipt for optimum stability. Repeated freeze/thaw cycles should be avoided.

Shipping Conditions
Dry ice or blue ice.

Unit Definition
One unit is defined as the amount of enzyme that incorporates 10 nmol of dNTPs in 30 minutes at 72°C.

5. Reaction Set Up (Standard reaction, 50 µl volume)

Once thawed, keep all reagents on ice and mix as described in a nuclease-free tube.

5x HiFi Buffer	10 µl
10 mM dNTP Mix (2.5 mM each)	5 µl
Template	X µl
Primers (20 µM each)	1 µl
PRECISOR High-Fidelity DNA Polymerase	1 µl
DMSO (optional)	1.5 µl
Water (ddH_2O)	up to 50 µl

Important: Due to the 3'-5' exonuclease activity inherent to PRECISOR High-Fidelity DNA Polymerase, the enzyme must be added last to a reaction in order to prevent primer degradation.

5.1 Standard Cycling Conditions

Cycle Step	Temperature	Time	Cycle(s)
Initial denaturation*	98°C	2 min	1
Denaturation	98°C	30 s	20-35
Annealing**	X°C (X: 2-5°C below the melting temperature of the lower T_m primer)	30 s	
Extension***	72°C	15-30 s/kb	
Final Extension (optional)	72°C	4-10 min	1
Cooling	4°C		

*__Initial denaturation__:
It is possible to elongate the initial denaturation at 98°C to up to 5 min for very complex templates such as chromatin.

**__Annealing__:
It is recommended to start with an annealing temperature of 55°C. If necessary, run a temperature gradient to determine the optimal annealing temperature.

***__Extension__:
Use extension time of 15 s per 1 kb for plasmid templates up to 5 kb.
Use extension time of 30 s (up to 1 min) per 1kb for plasmid templates >5 kb and for genomic DNA.

Note: Cycling conditions may need optimization in some cases.

5.2 General Considerations

Mg^{2+} Concentration:
When set up as described in 5., the reaction contains 2 mM Mg^{2+} (final concentration).
In some cases optimization may be necessary to achieve the best possible results, therefore 50 mM $MgCl_2$ Solution is provided in a separate tube.
The optimal Mg^{2+} concentration depends on the dNTP concentration used. In general a 1:2 ratio (dNTP:Mg^{2+}) is regarded as optimal, resulting in a final Mg^{2+} concentration of 2mM when using the reaction set up described in 5. Nevertheless, some optimization may be necessary, especially if using dNTP concentrations higher than the recommended one. Non-optimal concentration of Mg^{2+} leads to inefficient dNTP incorporation. 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.

Enzyme:
We recommend a range of 0.5–2.0 units of PRECISOR High-Fidelity DNA Polymerase in a 50 µl reaction.
Do not exceed 2u/50µl.

dNTP concentration:
We recommend a final concentration of 250 µM of each dNTP. Do not use dUTP or dITP.

Buffers and DMSO:
Two buffers are available: 5x HiFi Buffer and 5x GC Buffer.
The default buffer is the 5x HiFi Buffer which has been designed to provide high yields and high fidelity for the majority of standard templates.
For difficult templates such as genomic DNA or those possessing complex structural organisation, results may be enhanced by the addition of 1.5 µl DMSO per 50 µl reaction (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.
The 5x GC Buffer has been specifically designed for GC-rich templates and should only be used for templates with very high GC content (>65%), or as an alternative option in the event that the combination of HiFi Buffer and DMSO did not lead to satisfactory results.

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

Template:
The amount of template in the reaction depends mainly on the type of DNA used.
For shorter templates up to 5kb such as plasmid DNA or λ genomic DNA, we recommend using 50 pg-10 ng DNA per 50 µl reaction volume.
For larger templates such as mammalian genomic DNA we recommend a starting amount of 200 ng DNA per 50µl reaction, this may be varied between 5 ng-500 ng.
Furthermore, it is important to avoid, whenever possible, templates that are resuspended in EDTA-containing solutions (e.g. TE buffer) since EDTA chelates free Mg^{2+}.

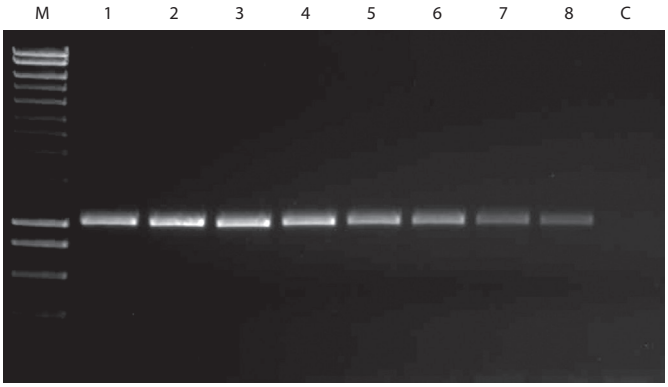
Templates with high GC content:
For the amplification of fragments with a GC content higher than 65% we recommend to use the GC Buffer.

6. Fidelity of PRECISOR High-Fidelity DNA Polymerase

The fidelity of PRECISOR High-Fidelity DNA Polymerase was determined in direct comparison with the leading high-fidelity enyzme using the *rps*L fidelity assay (Lackovich et al., 2001; Fujii et al., 1999) and found to be slightly higher.

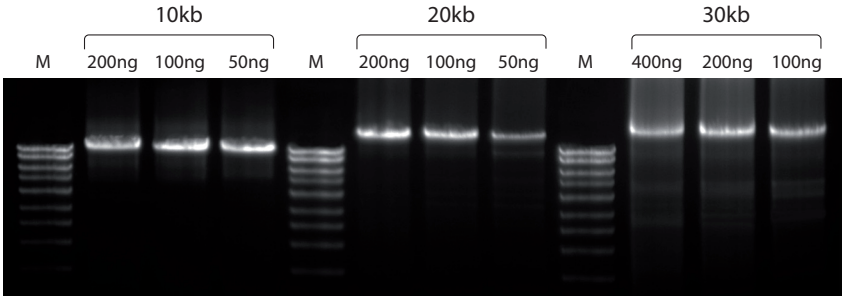
7. Performance of PRECISOR High-Fidelity DNA Polymerase

7.1 High-yield amplification of low template amounts with PRECISOR High-Fidelity DNA Polymerase



Amplification of low amounts of a 1kb genomic DNA fragment using PRECISOR High-Fidelity DNA Polymerase results in high yields.
A 1kb fragment of the mouse 18S rRNA gene was amplified using 1.5 units of PRECISOR High-Fidelity DNA Polymerase and HiFi Buffer. Starting template amount was 6.25ng mouse genomic DNA (lane 1), followed by a series of 2-fold dilutions (lanes 2-8). PCR was performed in 50µl reaction mixtures containing 2mM $MgCl_2$. Extension time was 30s/kb. M: DNA Ladder; C: Non-template Control.

7.2 Fast amplification of large genomic DNA fragments using PRECISOR High-Fidelity DNA Polymerase



Only 20 PCR cycles are required for amplification of large genomic DNA fragments using PRECISOR High-Fidelity DNA Polymerase.
Indicated starting amounts of 10, 20, and 30kb-sized Lambda DNA fragments were amplified using 2 units of PRECISOR High-Fidelity DNA Polymerase and 20 PCR cycles. PCR was performed in 50µl reaction mixtures with GC Buffer and a final $MgCl_2$ concentration of 2 mM. Extension time was 30s/kb. M: DNA Ladder.

8. Trouble Shooting

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 concentration 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; start with 3% final concentration and increase it up to 10% if necessary Try the GC buffer instead of the HiFi buffer
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	Successively replace each component in order to find the possible source of contamination Separate the areas for setting up the PCR reaction and for analyzing the PCR product

9. Related Products

Description	Cat#	Size	www.biocat.com/
HighRanger 1kb DNA Ladder (300bp - 10,000 bp)	11900-NB	100 loads	qdnamarkers
PCR Ranger 100bp DNA Ladder (50bp - 1,000 bp)	11300-NB	100 loads	qdnamarkers
PCR Sizer 100bp DNA Ladder (100bp - 1,000bp)	11400-NB	100 loads	qdnamarkers

10. Scientific Support

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For additional information or technical assistance, please call or email us at:

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