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Verbatim High Fidelity DNA Polymerase

Description: Verbatim High Fidelity DNA Polymerase is a novel, single enzyme system that gives an industry leading performance when compared to other high fidelity polymerases and polymerase blends. The intrinsic processivity of Verbatim results in significant improvements in yield, speed, target length and the ability to amplify difficult templates. Verbatim exhibits superior fidelity (3'-5' exonuclease activity) compared to all standard proofreading enzymes. Verbatim is recommended for amplicons up to 7kb from plasmid or lambda DNA and up to 5kb from genomic DNA.

Kit Contents:

Vial (cap color)	Pack Size	
	A	B
Verbatim High Fidelity DNA Polymerase (clear)	100 µl	5 x 100 µl
5X High Fidelity Buffer with $MgCl_2$[1] (red)	1 ml	5 x 1 ml
5X GC Buffer with $MgCl_2$[1] (green)	1 ml	5 x 1 ml
25 mM $MgCl_2$(clear)	150 µl	1 x 750 µl

[1]Both buffers contain $MgCl_2$ at a concentration of 2 mM

Verbatim High Fidelity DNA Polymerase includes two buffers for optimal performance with both standard and difficult templates. Both buffers contain magnesium chloride for convenient reaction setup.

Storage Conditions: Store at -20°C in a constant temperature freezer for up to 1 year. Avoid freeze thawing. Shipped on ice within the UK and on dry ice for international and within the US.

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Reaction Setup:

1) Just prior to thermal cycling combine the following reaction components on ice – adding Verbatim High Fidelity DNA Polymerase after all other reaction components:

Reaction Component	Final Volume
5x High Fidelity or GC Buffer*	5 µl
20 mM dNTP Mix	1.5 µl
Forward Primer (10µM; final concentration 0.3 µM)	0.75 µl
Reverse Primer (10µM; final concentration 0.3 µM)	0.75 µl
Template DNA	As required
PCR Grade Water	Up to 25 µl
Verbatim High Fidelity DNA Polymerase (1U/µl in storage buffer)	0.5 µl
Total Volume	**25.0 µl**

*Please note, Verbatim High Fidelity Buffer is recommended for most assays. GC Buffer is recommended for difficult templates with high GC content or assays where Fidelity Buffer produces low yield.

The standard reaction conditions described above will give a high-yield of specific PCR product in most instances. If optimization is required, the concentration of Verbatim should be varied between 0.25 U and 1 U in a 25 µl reaction. Increasing the concentration of $MgCl_2$, template and/or primers may also give improved results. Both buffers contain $MgCl_2$ at a final concentration of 2 mM. However, additional $MgCl_2$ may be required for some primer/template combinations. The optimal $MgCl_2$ for each specific application can be determined by increasing the final $MgCl_2$ concentration in 0.25 mM increments. It is recommended to perform both positive and negative (no template) control reactions in order to validate your PCR results.

2) Vortex briefly and centrifuge briefly to collect reaction components in the bottom of the tube

3) Load the reactions on to a thermal cycler and start the protocol immediately. Please refer to the cycling parameters described below for our recommended reaction guidelines.

Protocol Guidelines:

The table below summarises the thermal cycling conditions that give high yield, specific results in most instances.

PCR Step	Temperature	Duration	No. of Cycles
Initial Denaturation	95°C	2-5 mins	1 cycle
Denaturation	98°C	20s	15-35 cycles
Annealing	Tm +/- 10°C	15s	
Extension	72°C	30s/kb	
Final Extension	72°C	1-5mins	1 cycle

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Further Recommendations:

Amplicon length

Verbatim is recommended for high-yield amplification of targets up to 7 kb. For the efficient amplification of larger fragments, higher template concentrations and optimization of enzyme and $MgCl_2$ concentration may be required.

Template DNA

Amplification from low complexity templates such as lambda or plasmid DNA is often straightforward and generally requires little optimization. Amplification of low copy number targets are generally more challenging. For plasmid or phage DNA, 5 ng template per 25 µl reaction is adequate, whereas up to 50 ng genomic DNA or cDNA may be required.

Extension time

Verbatim is capable of amplifying targets up to X kb in length at an extension rate of 30 sec/kb. The duration of the extension step may need to be optimized for difficult templates. It may be possible to reduce extension times if sufficient template is present, while longer extension times (up to 1 min/kb) may give improved sensitivity and/or yield.

Primers and annealing temperature

Primer GC content should be approximately 40-60%. For GC-rich templates the GC Buffer is likely to give superior results. A GC content >60% may require a higher denaturation temperature and/or a longer denaturation time. Primer pairs should exhibit similar melting temperatures (Tm). Primers for two-step cycling programs should be designed with a high Tm value to ensure efficient annealing and extension at a single temperature (in the range of 65 - 72°C). As a first approach, use an annealing temperature equal to the lowest calculated Tm for the primer pair. To improve sensitivity, reducing the annealing temperature in increments of 1°C may give improved results. To improve specificity, increasing the annealing temperature in 1°C increments may be beneficial. It is recommended to determine the optimum annealing temperature empirically using an annealing temperature gradient.

$MgCl_2$ concentration

Both buffers contain $MgCl_2$ at a final concentration of 2 mM. However, additional $MgCl_2$ may be required for some primer/template combinations. The optimal $MgCl_2$ for each specific application can be determined by increasing the final $MgCl_2$ concentration in 0.25 mM increments.

TA cloning

DNA fragments generated with Verbatim are suitable for blunt-end cloning. For TA cloning, dA overhangs should be added to blunt-ended Verbatim PCR products using ThermoPrime DNA Polymerase in an A-tailing reaction. Following PCR amplification, Verbatim must be removed in a clean up reaction. The dA overhangs can then be added in an additional extension step at 72°C

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Ordering Information:

AB-1920/A	Verbatim High Fidelity DNA Polymerase	100 units
AB-1920/B	Verbatim High Fidelity PCR Polymerase	500 units

Troubleshooting:

For troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

For UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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