High Fidelity DNA Polymerase
Cat #: 42-500C

Unit size: 1000 Units
Buffers
5X High Fidelity Buffer 7.5 mM $MgCl_2$
50 mM $MgCl_2$ solution provided

Content: 2 x 500 Units

Concentration: 2 units/µl

Storage: -20°C.
Reagent for *in vitro* laboratory use only

General Description

Apex High Fidelity DNA Polymerase is a thermostable, chimeric DNA Polymerase created specifically for low-bias, high fidelity amplification of a vast range of amplicons. **Apex** High Fidelity DNA Polymerase delivers high-speed elongation and processivity, due to its fusion with a DNA-binding domain.

Key features

- High fidelity: > 60x Taq[1)]
- Long range amplification: 18 kb human genomic DNA and 25 kb for λ DNA
- High elongation rate: 10 sec/kb
- Excellent performance on a vast range of amplicons (High AT and high GC)
- Recommended for cloning, mutagenesis and molecular applications requiring extremely high fidelity

[1)] Determined through a novel NGS-based analysis of nucleotide misincorporation during PCR

Apex High Fidelity Storage Buffer

Enzyme is supplied in 50 mM Tris-HCl pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Tween® 20, 50% glycerol.

5 M Betaine Enhancer Solution

Sold separately. Cat No.: 42-504

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-precipitable form in 30 minutes at 72°C under standard assay conditions.

Quality Control

High Fidelity DNA Polymerase is tested for contaminating activities with no traces of endonuclease activity or nicking activity. Furthermore, long range capacity is tested on a human gDNA target of 18 kb.

Protocol

Optimal reaction conditions such as incubation times, temperatures and amount of template DNA may vary and must be determined individually. Amplification of templates with high GC content, extensive secondary structures as well as long range amplification may require more optimization - for tips see section *Strategies for Optimization*.

Prepare reaction mixtures in an area separate from that used for DNA preparation or product analysis. **Always work on ice.**

1. Thaw 5X High Fidelity Buffer, dNTP mix and primer solutions. **A precipitate is often seen in the 5x High Fidelity Buffer after thawing. It is recommended to completely thaw and thoroughly mix the buffer to ensure proper resuspension of precipitates.**
2. Prepare a master mix according to Table 1. The master mix typically contains all the components needed for amplification except the template DNA. It is important to add High Fidelity DNA Polymerase last to prevent primer degradation caused by the 3'→5' exonuclease activity.
3. Mix the master mix thoroughly and dispense appropriate volumes into reaction tubes. Mix gently, e.g., by pipetting the master mix up and down a few times.
4. Add template DNA to the individual tubes containing the master mix.
5. Program the thermal cycler according to Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
6. Place the tubes in the thermal cycler and start the reaction.

Table 1. Reaction components for a 25 µl reaction

Component	Vol./reaction	Final Conc.
5X High Fidelity Buffer	5 µl	1X
dNTP mix (10 mM of each)	0.5 µl	0.2 mM each dNTP
$MgCl_2$ (50 mM)	0 – 1.5 µl	1.5–4.5 mM
Primer A (10 µM)	0.5 µl	0.2 µM
Primer B (10 µM)	0.5 µl	0.2 µM
Apex High Fidelity DNA Polymerase	0.125 – 0.5 µl	0.25 – 1 unit
Betaine (5M)*	5 - 10 µl	1 - 2M
PCR Grade Water	Variable	- - - -
Template DNA	Variable	Variable
TOTAL volume	25 µl	- - - -

* Suggested for GC-rich amplification and long-range amplification. See section *Strategies for Optimization.*

Strategies for PCR Optimization

Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.

- Increased amount of Apex High Fidelity DNA Polymerase (up to 1U) often resolves low-yield reactions from very long targets (>8 kb)
- Increased dNTP concentration (up to 1.6 µM) often increases yield and decreases unspecific product creation.
- The addition of 1-2 M Betaine solution often improves reaction performance
- Increased template concentration will increase product yield.
- Increased primer concentration can increase product yield for some reactions.

GC-rich amplification

- The addition of 1 - 2 M Betaine solution often improves reaction performance

Primers

- Primers of 20 – 40 nucleotides with a GC content of 40 - 60 % are recommended.

$MgCl_2$

- The optimal $MgCl_2$ concentration should be determined empirically but in most cases a concentration of 1.5 mM, as provided in the common 1X High Fidelity Buffer, will produce satisfactory results. Table 3 provides the volume of 50 mM $MgCl_2$ to be added to the master mix if a higher $MgCl_2$ concentration is required.

Table 2. Three-step PCR program for targets < 1kb

Cycles	Duration of cycle	Temperature
1	2 min[a)]	98 °C
25 – 35	10 – 20 sec[a)] 15 – 30 sec[b)] 10 – 60 sec[c]	98 °C 55 – 70 °C 72 °C
Final elongation	5 min	72 °C

a. Denaturation: 2 min initial denaturation is sufficient for most templates. During thermocycling, 10 seconds usually works very well. Longer denaturation times might be required for long range PCR or amplification from templates with a high GC content.

b. Primer annealing: Typically, the annealing temperature is about 3 – 5 °C below the T_m (melting temperature) of the primers used. **Because of the high salt content within the 5X High Fidelity Buffer, annealing temperature will likely be higher than with more traditional PCR buffers.**

c. Extension: The recommended extension temperature is 72°C. Extension times highly depends on the length of the amplicon. **Generally, we recommend an extension time of 10-30 seconds per kb for complex genomic targets. 10 seconds per kb is often sufficient for simpler targets (such as plasmid) or short complex targets (< 3 kb). 30-60 seconds per kb is recommended for long amplicons (> 3 kb).**

Table 3. $MgCl_2$ concentration in a 25 µL reaction

Final $MgCl_2$ conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 50 mM $MgCl_2$	**0**	**0.25**	**0.5**	**0.75**	**1.0**	**1,25**	**1.5**

Recommended Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
Optional: Store at +4 °C for up to 6 months.

Related Products

Taq Polymerase kits (500 units)	Cat#
With 10X Standard and Ammonium Reaction Buffer	42-800B1
With 10X Combination Buffer	42-800B3
Glycerol Free	42-800B4

Hot Start DNA Polymerase kit (500 units)	Cat#
With 10X Ammonium and Combination Reaction Buffer	42-106

High Fidelity DNA Polymerase (500 units)	Cat#
With 5X High Fidelity Reaction Buffer	42-500B

Master Mixes (500 reactions)	Cat#
2X Taq RED Master Mix, 1.5 mM $MgCl_2$	42-138
2X Taq Master Mix, Clear, 1.5 mM $MgCl_2$	42-134
2X Hot Start Master Mix Buffer I, 1.5 mM $MgCl_2$	42-198
2X Hot Start Master Mix Buffer I Blue, 1.5 mM $MgCl_2$	42-144
2X High Fidelity Master Mix	42-501B

Real-time PCR (400 reactions)	Cat#
qPCR 2X Master Mix for Probe, without ROX™	42-116P
qPCR 2X Master Mix for Probe, low ROX™	42-118P
qPCR 2X Master Mix for Probe, high ROX™	42-120P
qPCR 2X GREEN Master Mix, without ROX™	42-116PG
qPCR 2X GREEN Master Mix, low ROX™	42-118PG
qPCR 2X GREEN Master Mix, high ROX™	42-120PG

Extraction Solution (500 reactions)	Cat#
DNA Extraction Solution, 500 reactions	42-503B

Genotyping PCR kit (500 reactions)	Cat#
Extract-Amp RED PCR Kit, 500 reactions	42-502B

Ultrapure dNTPs	Cat#
dNTP set, 100 mM each: 250 µl of each dA, dC, dG and dT	42-410
dNTP Mix 40 mM (1 x 500 µl): 10 mM each dA, dC, dG, dT	42-411

DNA Ladders	Cat#
Apex 100 bp-Low DNA Ladder, 250 applications	19-109
Apex 1 kb DNA Ladder, 333 applications	19-115
Apex 200 bp DNA Ladder, 200 applications	19-111
ECON Mini DNA Ladder 100-500 bp, 100 applications	19-130
ECON Low DNA Ladder 100-1000 bp, 100 applications	19-131
ECON PCR Ladder 100-3000 bp, 100 applications	19-132

Accessory reagents	Cat#
50 mM MgCl2, 3 × 1.5 ml	42-303
Nuclease-Free Water, PCR Grade, 6 x 5 ml	42-710