AK Taq DNA Polymerase V2

Cat. No.: FP1508925 | Pack size: 250 U / 1 KU / 2.5 KU / 4x2.5 KU | Storage: -20 °C; avoid repeated freeze/thaw

Overview

AK Taq DNA Polymerase V2 is developed based on wild-type Taq DNA polymerase. Genetic engineering modification improves its binding ability to DNA templates and enhances resistance to inhibitors in PCR templates, allowing amplification when conventional wild-type Taq polymerase may fail.

The enzyme retains the 5'->3' exonuclease activity of Taq polymerase and is suitable for probe-based real-time PCR (qPCR). Two anti-Taq monoclonal antibodies are included to block different active sites before high-temperature activation, improving specificity and reducing primer/probe degradation in premixed systems.

Polymerase activity is restored after heating at 95 °C for 2.5 min. With the improved buffer system, this product supports conventional PCR, qPCR, rapid PCR, direct whole-blood amplification, and amplification from samples containing elevated levels of interfering substances such as blood, soil, plant sections, and sputum.

Key Features

- Hot-start Taq polymerase with antibody-mediated activity blocking before activation
- Enhanced template binding and anti-interference performance for difficult PCR samples
- Retains 5'->3' exonuclease activity for probe-based qPCR detection
- Rapid activation under conventional PCR conditions; no special inactivation step required
- Applicable to hot-start PCR, direct whole-blood amplification, anti-interference amplification, and rapid PCR

Contents & Storage

Cat. No.	Component	250 U	1 KU	2.5 KU	4x2.5 KU	Storage
FP1508925A	AK Taq DNA Polymerase V2 (5 U/μL)	50 μL	200 μL	500 μL	4x500 μL	-20 °C; avoid freeze/thaw
FP1508925B	5x AK Taq Buffer (without Mg^{2+})	1 mL	4x1 mL	10x1 mL	40x1 mL	-20 °C; avoid freeze/thaw
FP1508925C	100 mM $MgCl_2$	100 μL	400 μL	1 mL	4x1 mL	-20 °C; avoid freeze/thaw

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Template DNA	Mammalian genomic DNA, E. coli genomic DNA, whole blood, or validated sample lysate	PCR/qPCR template
Primers and probe	Sequence-specific primers; TaqMan probe for qPCR	Target amplification/detection
dNTP Mix	25 mM each, molecular biology grade	PCR substrate
Nuclease-free water	DNase/RNase-free	Reaction-volume adjustment
PCR/qPCR tubes or plates	Compatible with instrument	Thermal cycling
Thermal cycler or qPCR instrument	Programmed for recommended cycling conditions	Amplification and fluorescence acquisition
Ice bath or ice box	Clean and nuclease-free workflow	Reaction setup

Preparation Before Use

1. Thaw and mix all reaction solutions at room temperature or 4 ℃, then place them on ice before use.
2. Aliquot reaction solutions whenever possible to avoid repeated freeze/thaw cycles.
3. Prepare PCR/qPCR reactions on ice or in an ice box. Mix gently and collect liquid at the bottom of each tube by brief centrifugation before cycling.

Protocol

Primer Design Guidelines

1. The last base at the 3' end of the primer is preferably G or C.
2. Avoid continuous mismatches in the last 8 bases at the 3' end of the primer.
3. Avoid hairpin structures at the 3' end of the primer.
4. The Tm difference between the forward and reverse primers should preferably not exceed 1 ℃; a Tm of 55-65 ℃ is recommended.
5. Do not include non-template-paired additional sequences when calculating primer Tm.
6. Control primer GC content between 40% and 60%.
7. Distribute A, G, C, and T as evenly as possible and avoid extremely GC-rich or AT-rich regions.

8. Avoid complementary sequences longer than 5 bases within one primer or between primers; avoid complementary sequences longer than 3 bases at the primers' 3' ends.
9. Check primer specificity with NCBI BLAST after design to reduce non-specific amplification.

qPCR Reaction Setup

Component	Volume Added	Final Concentration
5x AK Taq Buffer (without Mg^{2+})	10 µL	1x
dNTP Mix (25 mM each)	0.4 µL	0.2 mM each
Forward Primer (10 µM)	1 µL	0.2 µM
Reverse Primer (10 µM)	1 µL	0.2 µM
Probe (10 µM)	0.5 µL	0.1 µM
AK Taq DNA Polymerase V2 (5 U/µL)	0.5 µL	0.05 U/µL
100 mM $MgCl_2$	1 µL	2 mM
Template DNA	10 µL	—
ddH_2O	To 50 µL	—
Final volume	50 µL	—

The working concentration of $MgCl_2$ can be adjusted within 1-3 mM. Excessively high Mg^{2+} concentration may increase non-specific products.

Recommended template inputs: mammalian genomic DNA, 0.1-1 µg; E. coli genomic DNA, 10-100 ng.

For qPCR with whole blood, keep the volume of whole blood at no more than 5% (v/v) of the reaction volume to avoid fluorescence-signal interference.

For rapid qualitative amplification of long fragments, especially fragments >=1 kb, 5x AK Taq Fast Buffer is recommended. 5x AK Taq Fast Buffer is not recommended for qPCR because it may reduce fluorescence signal.

Conventional Qualitative PCR

Step Name	Temperature	Time	Number of Cycles
Heat Shock	95 °C	2 min 30 s	1
Denaturation	95 °C	10 s	30-35
Annealing	55 °C	20 s	30-35

Step Name	Temperature	Time	Number of Cycles
Extension	72 ℃	20 s	30-35
Final Extension	72 ℃	7 min	1
Storage	12 ℃	∞	1

Rapid PCR (1 kb λDNA Example)

Step Name	Temperature	Time	Number of Cycles
Heat Shock	95 ℃	2 min 30 s	1
Denaturation	98 ℃	1 s	30-35
Annealing	55 ℃	5 s	30-35
Extension	72 ℃	5 s	30-35
Final Extension	72 ℃	5 min	1
Storage	12 ℃	∞	1

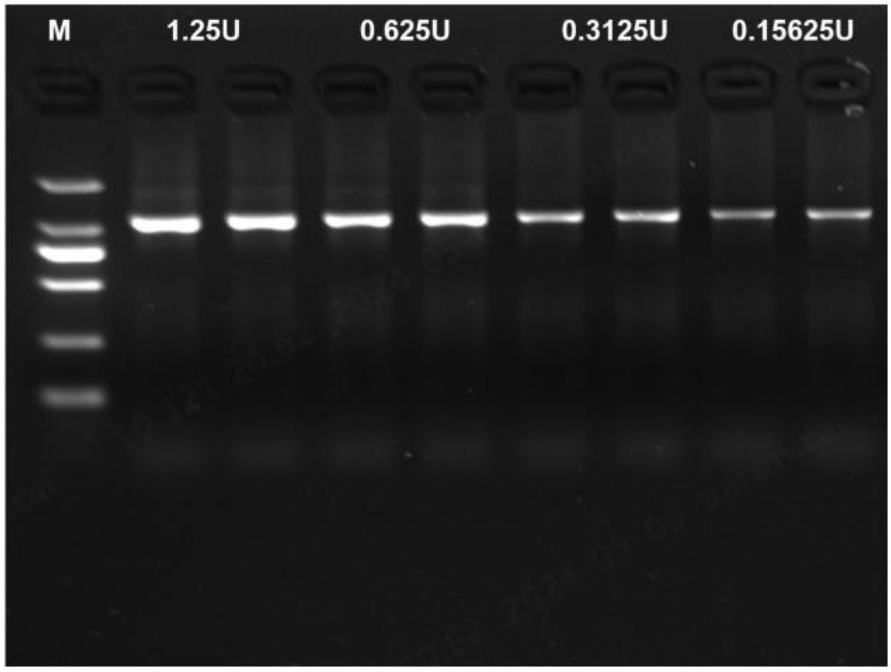
Fluorescent Quantitative PCR

Step Name	Temperature	Time	Number of Cycles
Heat Shock	95 ℃	2 min 30 s	1
Denaturation	95 ℃	15 s	35-40
Annealing + Extension	55 ℃	40 s	35-40

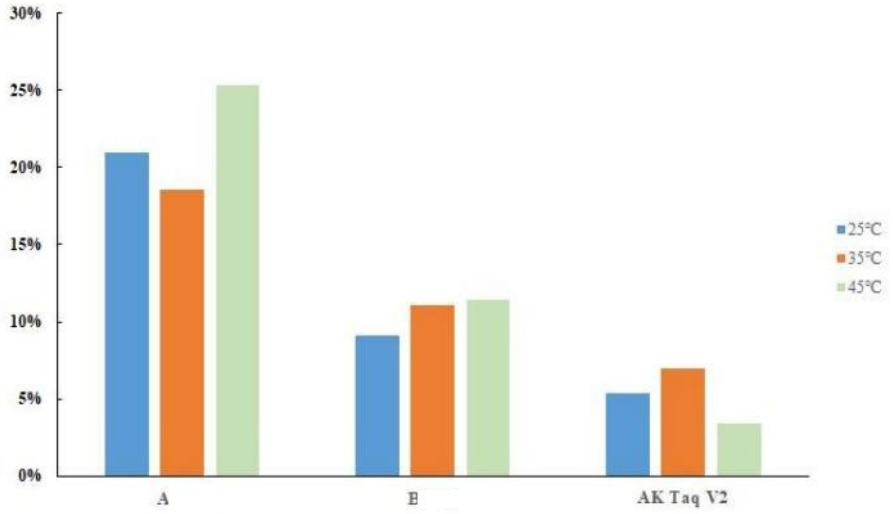
PCR settings should be optimized according to template, primer design, product length, and GC content.

As a starting point, use approximately 5 s extension time per kb for rapid amplification. If results are not satisfactory, extend to 10-15 s per kb or optimize cycling conditions.

Experimental Examples



Experimental example: AK Taq V2 extension-rate performance with short extension time.



Experimental example: AK Taq V2 blocking effect on 5'->3' exonuclease activity under different temperature conditions.

AK Taq V2 shows strong extension performance. When the extension time is 5 s and the enzyme amount is as low as 0.15625 U, it can still amplify a 1 kb fragment.

Under different temperature conditions, AK Taq V2 has an improved blocking effect on 5'->3' exonuclease activity and can reduce degradation of primer-probes.

Storage & Handling

Store all components at -20 °C. Upon receipt, aliquot when appropriate and avoid repeated freeze/thaw cycles.

Keep enzyme solutions on ice during reaction setup and return them to -20 °C immediately after use.

Safety & Precautions

1. Excessive template DNA may lead to non-specific PCR products; optimize template input for each sample type.
2. Use nuclease-free tubes, tips, and water to prevent nucleic acid degradation or contamination.
3. Wear lab coat, disposable gloves, and eye protection. Read the current SDS before use.

Quality Control

QC Item	Method	Acceptable Range
Component completeness	Visual inspection on receipt	All listed components present; no leakage or visible damage
Enzyme performance	Functional amplification or end-repair assay	Meets product specification when used under recommended conditions
Storage integrity	Cold-chain and label check	Stored at the specified temperature and protected from repeated freeze/thaw cycles

Troubleshooting

Issue	Possible Causes	Corrective Action
No or weak amplification	Insufficient template; inhibitors in sample; incorrect cycling program	Optimize template input, Mg^{2+} concentration, and cycling conditions; verify primer/probe design
Non-specific bands or primer dimers	Primer design issue; excessive template or Mg^{2+}	Redesign primers, reduce template amount, lower Mg^{2+}, or increase annealing temperature
Low qPCR fluorescence signal	Incompatible buffer or excessive blood/sample lysate	Use recommended qPCR conditions; keep whole blood <=5% of reaction volume

Recommended Applications

Hot-start PCR · direct whole-blood amplification · anti-interference amplification · rapid PCR · probe-based qPCR

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Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

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