INSTRUCTION MANUAL

【Product】

Product：AK Taq DNA Polymerase V2

Product No.：MDAD006

【Description】

AK Taq DNA Polymerase V2 is a genetically engineered DNA polymerase variant of Taq DNA Polymerase with enhanced binding ability to DNA substrates, giving it the ability to amplify PCR templates in the presence of inhibitors. The enzyme is suitable for probe-based quantitative PCR (qPCR) due to retaining 5'→3' exonuclease activity. AK Taq DNA Polymerase V2 is modified by dual Anti-Taq monoclonal antibodies with improved specificity.

Prior to heat-shock, one monoclonal antibody is bound to the Taq DNA Polymerase active site while the second is bound to the enzyme's exonuclease site, inhibiting both exonuclease and polymerase activity at ambient temperatures. This prevents non-specific amplification at ambient temperatures derived from mis-priming or due to formation of primer-dimers during reaction preparation. This also reduces degradation of primers and probes by Taq DNA polymerase in pre-mix solutions, which ensures the long-term stability of pre-mix products.

When amplification reactions are heated at 95°C for two and half minutes, polymerase activity is restored due to the denaturation of the Anti-Taq monoclonal antibodies, yielding active polymerase that can be used directly under standard PCR reaction conditions. In combination with optimized buffer, PCR proceeds rapidly and effectively with superior sensitivity, specificity, and product yield. In addition, the product exhibits decreased inhibition when in the presence of blood, soil, plant material, sputum, and other common PCR inhibitors, and can be used for rapid PCR amplification protocols.

【Contents】

Components	MDAD006 -50	MDAD006 -200	MDAD006 -500
AK Taq DNA Polymerase V2 (5 U/μL)	50μL	200μL	500μL
5×AK Taq Buffer (without Mg^{2+})	1mL	1mL×4	1mL×10
5×AK Taq Fast Buffer (without Mg^{2+})	1mL	1mL×4	1mL×10
100mM $MgCl_2$	100μL	400μL	1mL

【Storage】

-15℃~ -25℃ for 2 years. Transport at -20±5℃.

【Applications】

Hot-start PCR, direct PCR amplification from whole blood, PCR amplification in the presence of inhibitors, fast PCR

【Unit definition】

1 unit refers to the amount of enzyme required to catalyze 10 nmol dNTP added into acid-insoluble precipitate at 74°C for 30 min using activated salmon sperm DNA as a template / primer.

【Notes】

Primer design guidelines

1 Ensure the 3' end of a primer ends in G or C to promote binding.

2 Avoid consecutive mismatches in final 8 bases at the 3' end of a primer.

3 Avoid hairpin structure formation at the 3' end of a primer.

4 Aim for the melting temperature (Tm) of the primers to fall between 55°C and 65°C, and within 1°C of each other (use Primer Premier 5 to calculate Tm).

5 Sequences in the primer that do not pair with the template should be excluded from the Tm calculation.

6 Aim for the GC content to be between 40 and 60%.

7 Avoid GC-rich and AT-rich regions and ensure that primers

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Mammalian Genome DNA	0.1-1μg
E. coli Genome DNA	10-100ng

have a balanced distribution of A, G, C, and T.

8 Avoid intra-primer homology (more than 5 bases that complement within the primer) or inter-primer homology (more than 5 complementary bases between primers or more than 3 complementary bases at the 3'-ends of primers).

9 Perform a specificity check of primers with NCBI BLAST.

【Other notes】

1 Excessive template DNA can lead to nonspecific PCR products.

2 It is recommended to use 5×AK Taq Fast Buffer (without Mg^{2+}) for qualitative rapid amplification of long fragments (≥1 kb).

【Protocol】

Reaction setup：

1 Thaw (room temperature/4℃) and mix well all reagents prior to use. Thawed reagents should be kept on ice. It is advised to aliquot reagents and avoid repeated freeze-thaw cycles.

2 In a PCR tube or PCR plate, combine the reaction components as directed in the table below for a 50μL reaction. Perform all reaction preparations on ice：

Component	Volume	Final Concentration
5×AK Taq Buffer (without Mg^{2+}) [d]	10μL	1×
dNTP (25mM each)	0.4μL	0.2mM
Forward Primer (10μM)	1μL	0.2μM
Reverse Primer (10μM)	1μL	0.2μM
Probe (10μM)	0.25μL	0.05μM
AK Taq DNA Polymerase V2 (5U/μL)	0.5μL	0.05U/μL
100mM $MgCl_2$ [a]	1μL	2mM
Template DNA [b, c]	10μL	—
ddH_2O	Up to 50μL	—
Total	50μL	—

a. The concentration of $MgCl_2$ can be adjusted between 1 and 3mM. Excessive Mg^{2+} is likely to lead to nonspecific amplification.

b. Recommended template amounts for a 50 μL reaction mix are as follows：

c. For quantitative PCR, the volume of whole blood added into the reaction should be less than 5% (v/v); excessive addition of whole blood samples will affect the collection of fluorescence signals.

d. It is recommended to use 5×AK Taq Fast Buffer for qualitative rapid amplification of long fragments (≥1 kb). However, the buffer can lead to decreased fluorescence signal and is not recommended for quantitative PCR.

【Thermocycling protocol】

1 Routine PCR：

Step	Temperature	Time	Cycles
Heat shock	95℃	2min30sec	1
Denaturation	95℃	10sec	30~35
Annealing	55℃	20sec	
Extension	72℃	20sec	
Final Extension	72℃	7min	1
Hold	12℃	∞	1

2 Fast PCR (for 1kb-lDNA):

Step	Temperature	Time	Cycles
Heat shock	95℃	2min30sec	1
Denaturation	98℃	1sec	30~35
Annealing	55℃	5sec	
Extension	72℃	5sec	
Final Extension	72℃	5min	1
Hold	12℃	∞	1

3 Quantitative PCR：

Step	Temperature	Time	Cycles
Heat shock	95℃	2min30sec	1
Denaturation	95℃	15sec	35~40
Annealing/Extension	55℃	40sec	

3.1 PCR conditions such as temperature, time, and cycling

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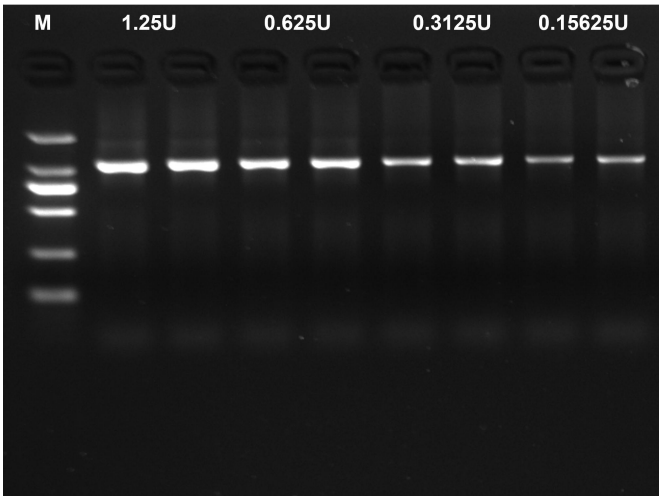
frequency may be adjusted according to the template, primer, PCR product length, and GC content.

3.2 Extension time should be set according to the length of the PCR product, typically allowing a minimum of 5 seconds per kb of product. For example, if the size of the amplicon is 1 kb, the extension time can be set to 5 seconds; if it's 2 kb, it can be set to 10 seconds. If needed, it can be further extended to 15 seconds, and similarly adjusted for other amplicon sizes.

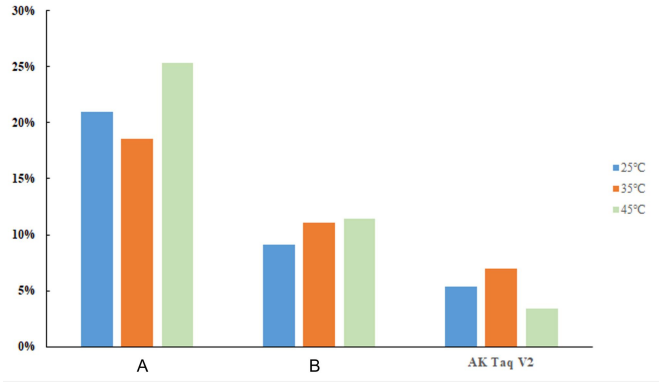
【Experimental examples】

Extension time: 5s



As low as 0.15 units of AK Taq DNA Polymerase V2 can amplify a 1kb target with an extension time of 5 seconds (25μL reaction).

Inhibition of 5'-3' exonuclease activity：



Inhibition of AK Taq V2's 5'-3' exonuclease activity is more potent than competitor products under various tested temperatures, an advantage which can effectively reduce the degradation of primers and probes.

【Related Products】

AK Taq DNA Polymerase V2.3 (Product No.：MDAD040)

【Manual Approval/Edit Date】

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