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For the Life Scientist

Hot AppTaq Polymerase

CONCENTRATION: 5U/µl

Store at -20°C. (The kit will retain full activity for 12 months at -20°C. Can be stored at 4°C for 1 month and go through 30 freeze/thaw cycles with no loss of activity. Avoid prolonged exposure to light).

DESCRIPTION

Hot AppTaq Polymerase is a high performance enzyme which has been specifically engineered for highly specific, hot-start PCR amplification of DNA fragments up to 6 kb. It remains inactive at ambient temperatures, minimising non-specific amplification and primer dimers during PCR set up. It is ideal for multiplex and colony PCR in high throughput applications. It comes with a 5x reaction buffer which has pre-added enhancers, stabilisers, $MgCl_2$ and dNTPs to maximise PCR yields, and works in fast or standard thermal cycling conditions. The enzyme generates 3'adenine overhangs on the PCR products which can then be cloned into TA vectors.

ORDERING INFORMATION

Component	ARP021	ARP023
Hot AppTaq Polymerase (5U/µl)	250 units (1x 50µl)	1000 units (4x 50µl)
5x Appleton reaction buffer	2x 1ml	8 x 1ml

PROTOCOL

Prepare a PCR master mix by mixing molecular biology grade water, 5x Appleton reaction buffer, forward and reverse primers and Hot AppTaq Polymerase. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes / wells and then add template DNA.

1. Gently mix and briefly centrifuge all solutions after thawing.

2. Add the following components for each 50µL reaction to a thin-walled PCR tube/plate:

Reagent	Final Concentration	50µL reaction
5x Appleton reaction buffer	1X	10.0µL
Forward primer (10µM)	400nM	2.0µL
Reverse primer (10µM)	400nM	2.0µL
Template DNA	100 - 500ng*	variable
Hot AppTaq Polymerase (5U/µL)	1.25U - 5U	0.25µL - 1µL
Molecular Biology Grade water, (BMW001)		Up to 50µl final volume

3. Gently mix the samples and spin down.

4. If using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 25µL of mineral oil.

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5. Perform PCR using recommended thermal cycling conditions:

Step	Temperature / °C	Time	Number of cycles
Initial denaturation and enzyme activation	95	1-2 min	1
Denaturation	95	15 s	25-40
Annealing	55-65	15 s	
Extension	72	1-90 s (15 s per kb)	

CONSIDERATIONS

Template DNA*

For optimal results, use between 5ng and 500ng per reaction for eukaryotic DNA, and for cDNA use below 100ng in the 50 µL reaction volume. Higher amount of template increases the risk of non-specific PCR products. Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

Reaction Buffer

The 5x Appleton reaction buffer contains optimal concentrations of $MgCl_2$ (15mM) and dNTPs (5mM), as well as enhancers and stabilisers. This avoids having to vary these parameters to obtain maximum PCR yields. We do not recommend adding further enhancers or magnesium.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mis-priming and non-specific PCR products.

Denaturation

Complete initial denaturation of the template DNA is essential for efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient. For colony PCR, denature for 10 mins. For highly GC-rich templates, we recommend using Mega-App Polymerase (ARP031) which has been developed specifically for the amplification of extremely difficult templates.

Annealing

The optimal annealing temperature is 5°C lower than the melting temperature (Tm) of the primers. Incubation for 0.25-2 min is usually sufficient. However, if non-specific PCR products are obtained in addition to the expected product, the annealing temperature should be optimized by increasing it stepwise by 1-2°C.

Extension

The optimal extension temperature for Hot AppTaq Polymerase is 72°C. The recommended extension time is 30 s at 72°C for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 15 s / kb. For multiplex PCR, use 90 s extension per cycle, and for short PCR targets (<1kb) only 1 s / cycle is needed.

TROUBLE SHOOTING / TECHNICAL SUPPORT

For troubleshooting please visit www.appletonwoods.co.uk/PCRtroubleshooting.pdf for a trouble shooting guide on PCR. If this does not resolve your issues, please email technicalsupport@appletonwoods.co.uk with details of your: amplicon size, reaction setup, cycling conditions, gel images.

Notes: Hot AppTaq Polymerase has an error rate of 1 error per 2.0 x 10^5 nucleotides incorporated. Hot AppTaq Polymerase is for research use only.

ASSOCIATED PRODUCTS

Product	Pack Size	Product Code
Molecular Biology Grade Agarose	500g	AG001
AxyPrep Mag PCR clean up Kit	50mL, 1110 preps	AX402
Molecular biology grade water	500mL	BMW002

More pack sizes available at www.appletonwoods.co.uk