Mega-App Mix (2X)

PACK SIZE: 200 reactions, (5x 1mL)

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

Mega-App Mix is a superior performance hot-start polymerase ready-mix which has been specifically engineered for long range PCR amplification of DNA fragments up to 35 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 as a 2x Mega mix 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	ARP092
Mega-App Mix (2X)	200 reactions (5x 1ml)

PROTOCOL

Prepare a PCR master mix by mixing molecular biology grade water, Mega-App Mix (2X), forward and reverse primers. 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
Mega-App Mix (2X)	1X	25.0µL
Forward primer (10µM)	400nM	2.0µL
Reverse primer (10µM)	400nM	2.0µL
Template DNA	100 - 500ng*	variable
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.

5. Perform PCR using recommended thermal cycling conditions:

ARP092

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	10 mins (50 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 50µL 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.

Mega-App Mix

The Mega-App Mix (2X) contains optimal concentrations of $MgCl_2$ (6mM) and dNTPs (2mM), enhancers, stabilisers and Mega-App Polymerase. This avoids having to vary parameters to obtain maximum PCR yields. We therefore do not recommend adding further enhancers or magnesium to PCRs.

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.

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 Mega-App Polymerase is 72°C. The recommended extension time is 15 s per kb for amplification from eukaryotic DNA for PCR products <5kb. For larger products, (>5kb – 35kb) the extension time should be prolonged by 40-60 s / kb.

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: Mega-App Polymerase has an error rate of 1 error per 5.0 x 10^5 nucleotides incorporated. Mega-App Mix is for research use only.

ASSOCIATED PRODUCTS

Product	Pack Size	Product Code
Molecular Biology Grade Agarose	100g	AG002
Molecular Biology Grade Agarose	500g	AG001
AxyPrep Mag PCR clean up Kit	5mL, 110 preps	AX401
AxyPrep Mag PCR clean up Kit	50mL, 1110 preps	AX402
AxyPrep Mag PCR clean up Kit	250mL, 5550 preps	AX403
Molecular biology grade water	100mL	BMW001
Molecular biology grade water	500mL	BMW002

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