CleanAmp™ PCR 2X Master Mix

Catalog # L-5101

L-5101-100 (100 reactions)
L-5101-BK (Bulk amount)

CleanAmp™ PCR 2X Master Mix is an optimized, ready-to-use mix of CleanAmp™ dNTPs and *Taq* DNA Polymerase in reaction buffer for standard PCR. Simply add primers, template DNA and water.

QC Analysis

Functional Assay; Pass
Tested in standardized PCR assay for efficiency and specificity.

Handling & Use

Store at -20°C
Stable to 15 freeze-thaw cycles. Exposure to ambient temperatures during shipping does not adversely affect product performance.

Product released by Quality Assurance

Protocols

Endpoint PCR (25 µL)

1. Thaw CleanAmp™ PCR 2X Master Mix, primers and DNA template and place on ice.
   Note: Do not vortex CleanAmp™ PCR 2X Master Mix. Mix thoroughly by pipetting up and down and collect by pulse centrifugation.
2. Prepare a reaction mixture containing all components except for the DNA template. Add CleanAmp™ PCR 2X Master Mix , primers and sterile de-ionized water as shown in Table 1 into thin-walled PCR tubes. Keep on ice.
3. Mix the reaction mixture gently to protect the enzyme, by pipetting up and down. Do not vortex. Pulse spin if necessary.
4. Add the appropriate volume of template DNA to reach a reaction volume of 25 µL.
5. Pulse spin to remove bubbles and collect reaction solution at bottom of PCR tube.
6. Place the tubes into a thermal cycler with a heated lid and perform the appropriate cycling conditions for standard thermal cycling:
   95°C for 0-5 min
   [95°C for 10-40 sec; 48-60°C[1] for 1-30 sec; 72°C for 0.5-2 min[2]]
   30-40 cycles, 72°C for 10 min
7. Analyze an aliquot of the completed reaction by agarose gel electrophoresis.

Table 1

Component	Final Concentration (25 µL reaction)	Volume per reaction
CleanAmp™ PCR 2X Master Mix	1X	12.5 µL
Forward/Reverse Primer	50-500 nM	Variable
DNA Template	Variable	Variable
Sterile De-ionized Water	Up to 25 µL	Up to 25 µL
Total Volume (µL)	**25 µL**	**25 µL**

[1] The annealing temperature should be chosen for optimal PCR performance. Most primer design software recommends an annealing temperature. The annealing temperature can also be optimized experimentally by using a thermal cycler with gradient functionality or by performing sequential experiments in which the annealing temperature is varied.
[2] The extension time at 72°C is recommended to be 30-60 seconds per kb of target.

Real-Time PCR

CleanAmp™ PCR 2X Master Mix has been successfully adapted for real-time detection using intercalating dye and probe-based detection. Please refer to the instrument manufacturer for specific protocols.

Real-time PCR may be proprietary. No license is conveyed expressly or by implication to the purchaser by purchase of any TriLink BioTechnologies products.

See Reverse for Troubleshooting

Troubleshooting

Probable Cause	Suggestion(s)
No amplification product or low amplicon yield	
Insufficient activation of CleanAmp™ dNTPs during thermal cycling	Optimize the duration of the initial denaturation time to up to 10 minutes.
Thermal cycling protocol is not optimized	Increase extension time. Generally extension times should be 30-60 seconds per kb of target.
	Increase the number of thermal cycles in 5 cycle increments.
	Optimize annealing temperature.
Problem with reagents or reaction conditions	Prepare fresh reagents, including CleanAmp™ PCR 2X Master Mix and primers.
	Verify that template is good in quality and of sufficient quantity.
	Verify primer design to ensure adequate complementarity to the DNA target.
Non-specific product formation	
Excessive off-target primer extension	Titrate the concentration of the primers or template DNA.
Primer dimer formation	Reduce initial denaturation and denaturation times: 95°C for 0-5 min, [95°C for 10-20 sec, 48-60°C for 1-15 sec, 72°C for 0.5-2 min] 30-40 cycles, 72°C for 10 min Note: A zero initial denaturation time in primer/template systems prone to primer dimer formation may cause a slight delay in threshold cycle (Ct) or quantification cycle (Cq).
Mis-priming	Omit initial denaturation time and shorten annealing time: [95°C for 30 sec, 48-60°C for 1 sec, 72°C for 0.5-2 min] 30-40 cycles 72°C for 10 min

Contact

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