CleanAmp™ GC-Rich PCR 2X Master Mix

Catalog # L-5102

L-5102-100 (100 reactions)
L-5102-BK (Bulk amount)

CleanAmp™ GC-Rich PCR 2X Master Mix is an optimized, ready-to-use mix specifically designed for robust amplification of targets over 60% in GC content. It contains CleanAmp™ dNTPs with CleanAmp™ 7-deaza-dGTP and *Taq* DNA Polymerase in reaction buffer. 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 10 freeze-thaw cycles. Exposure to ambient temperatures during shipping does not adversely affect product performance.

Protocols

Endpoint PCR (25 µL)

1. Thaw CleanAmp™ GC-Rich PCR 2X Master Mix, primers and DNA template and place on ice.
   Note: Do not vortex CleanAmp™ GC-Rich 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™ GC-Rich 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 10 min
   [95°C for 40 sec; 48-60°C[1] for 1 sec[2]; 72°C for 1 min[3]]
   35-40 cycles, 72°C for 7 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™ GC-Rich PCR 2X Master Mix	1X	12.5 µL
Forward/Reverse Primer	50-500 nM	Variable
DNA Template[4]	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] Annealing time varies between thermal cyclers. For traditional thermal cyclers, a short, second annealing time provides the best specificity. For fast cyclers use a 10 second annealing time.
[3] The extension time at 72°C is recommended to be 30-60 seconds per kb of target.
[4] 0.2 ng/µL of Human Genomic DNA is used in control reactions.