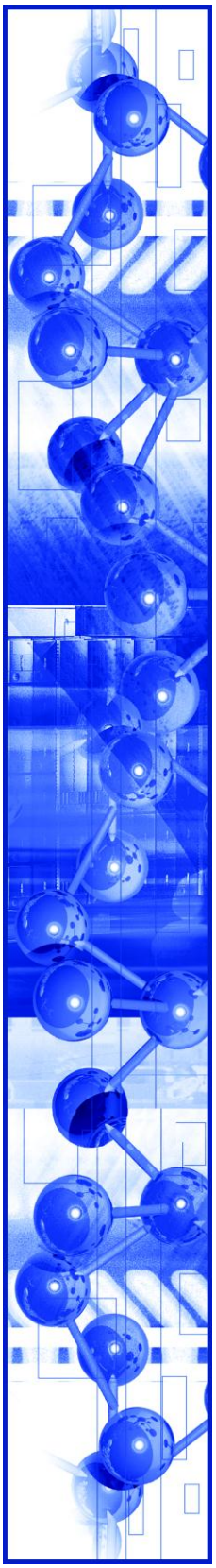


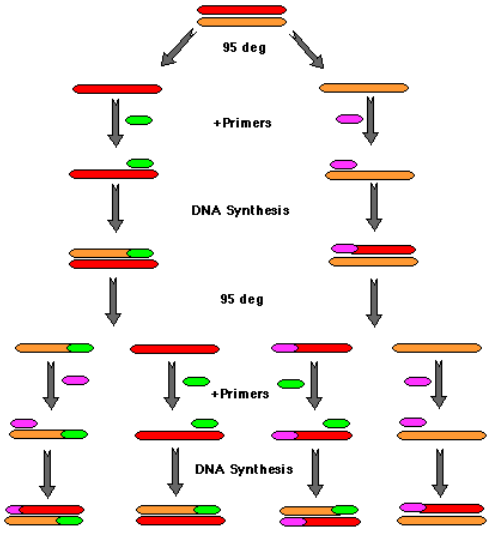
Taq Polymerase/ PCR Kit with Taq Polymerase

Code	Description	Size
N224-500U	TAQ DNA POLYMERASE, 500U	500 U
N555-KIT	PCR KIT WITH TAQ POLYMERASE	1 Kit

General Information

The PCR (Polymerase Chain Reaction) is a widely used molecular biology application for the rapid amplification (duplication) of specific gene sequences. The DNA to be amplified is added to a solution containing the following components: primers, dNTP's, DNA Polymerase, and reaction buffer containing divalent cation (Mg^{2+}, Mn^{2+}). A typical PCR reaction consists of three steps that combine to form one doubling cycle. In the first step, denaturation, the reaction is heated to 95ºC to relax the DNA template and separate the double-stranded helix. The reaction is then cooled to 35-72ºC for the second Annealing (binding) step which allows the primers to bind to the recognition sequence of the template. In the final, Elongation step, Taq polymerase extends from the 3' end of the primer at 72ºC to form a newly synthesized "daughter" strand. The cycle then repeats itself for a total of 25 – 50 times and results in an exponential increase in the concentration of double-stranded DNA template.

Figure 1. Exponential increase in double stranded DNA molecules by the Polymerase Chain Reaction.



1-800-448-4442

Storage/Stability:

Taq DNA Polymerase is stable for 1 year when stored frozen (-15 to -22ºC).

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Procedure:

***Notes**:*

- *Reaction solutions should be assembled on ice.*
- *Reaction volumes should not exceed 50 µl to ensure adequate thermal equilibration.*

1. Label thin-walled PCR tubes or a 96 well plate as needed.
2. To perform multiple, parallel reactions, prepare a master mix consisting of dNTPs, buffer ($MgCl_2$ if necessary), and polymerase. Consult Table I to determine the volume of each component for 50 µl reactions. Prepare at least 20% more master mix than actually needed to account for pipetting loses.

Table 1

Reaction Component	Volume
Template DNA	1-3µl (50-150ng)
Taq Buffer, 10X	5µl
dNTPs (25mM)	0.4 µl
Primer A (sense, 10µM)	4µl
Primer B (anti-sense, 10µM)	4µl
Sterile deionized water	As needed to bring final volume to 50µl
Taq polymerase 500U	0.5µl

3. Aliquot master mix to tubes or wells and add DNA templates and primers. See Table I for recommended volumes of each.
4. Gently vortex samples and briefly centrifuge to collect reaction mix in bottom of tube
5. Place in thermocyler and begin PCR. See Table II for cycling specifications. Cycling protocol may need to be optimized for each sample.

Table 2

Step	Temperature (ºC)	Time (minutes)	Number of cycles
Initial Denaturation	94 – 95	1 – 5	1
Denaturation	94 – 95	0.5	
Annealing	55 – 65 (depends on Tm of primers)	0.5 – 2.0	25 -35
Elongation	72	0.5 – 2.0	
Final Elongation	72	5 – 10	1

Notes*: During the final elongation step, the terminal transferase activity of Taq polymerase will incorporate an additional Adenosine nucleotide to the 3' ends of the resulting amplimers. If the PCR fragments are to be T/A cloned, this extension step can be extended for up to 30 minutes.*

Trouble Shooting

Symptoms	Possible Causes	Possible Remedy
Low or no yield	Insufficient number of cycles	Replace PCR vials in thermocycler and run an extra 5 cycles
	DNA template denatured	Check DNA quality on agarose gel
	Thermocycler program not correct	Check temp. and cycle times
	Inhibitors present in reaction	Precipitate the original samples or purify over columns. Ions such as EDTA may inhibit the PCR reaction.
	Missing reaction component	Check components and set up new reactions.
	Unsuitable reaction conditions	Decrease annealing temperature and/or increase elongation time.
	Evaporation	Cover reaction with high quality mineral oil or use a thermocycler with a heated lid.
	Bad primers	Insure that 5' and 3' ends of primers are not complementary. A high GC content or extremely mismatched Tm's may also have an effect on binding efficiency.
	Incorrect primer specificity	Check primer sequences
	Primer concentration to low	Check concentration and increase if necessary.
	Bad dNTPs	dNTP solutions should be stored frozen with limited freeze/thaw cycles. Replace nucleotide solution if necessary.
	Target not present in DNA template	Try another region in DNA template.
Non-specific Amplification	Sub-optimal reaction conditions	Optimize MgCl concentration, annealing temperature, elongation time, and number of cycles. Keep reactions on ice when all reagents are mixed.
	Badly defined primers	Re-design primers. Insure ends are not complementary and do not containg 3 successive G's or C's at 3' end.
	Primer concentration to high	Reduce primer concentration
	Contamination with other template	Use dedicated pipettes and tips. Work in separate rooms or clean areas.
	Several targets present with same target sequence	Develop new primers and run BLAST search in public database to insure primer specificity.

Related Products

Code	Product
E891	NucleasEliminator™
0710-500G	Agarose I™, 500 g General Use (also available as tablets, K857-100TABS)
0658-4L	TBE Buffer, 10X Liquid Concentrate
0796-1.6L	TAE Buffer, 25X Liquid Concentrate
N472, N650, N313	EZVision ® DNA Dye in Loading Buffer
K811-50RXN	PCR Marker with Loading Dye

Visit the AMRESCO website for additional related products
www.amresco-inc.com

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