PCR Kit with Taq Polymerase

Code	Description	Size
N555-KIT	**PCR Kit with Taq Polymerase**	200 Reactions

General Information

PCR (Polymerase Chain Reaction) is a widely used molecular biology application for the rapid amplification of specific gene sequences. The DNA to be amplified is added to a solution containing primers, nucleotides, DNA polymerase, and reaction buffer containing a 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, the reaction is heated to 95ºC to relax the DNA template and denature the double-stranded helix. The reaction is then cooled to 35 – 72ºC to enable annealing of the primers to the targeted sequence of the DNA template. In the subsequent elongation step at 72ºC, Taq polymerase catalyzes polymerization of nucleotides from the 3' end of the primer to form a newly synthesized "daughter" strand. The cycle is then repeated 25 – 50 times and results in an exponential increase in the concentration of double-stranded DNA template.

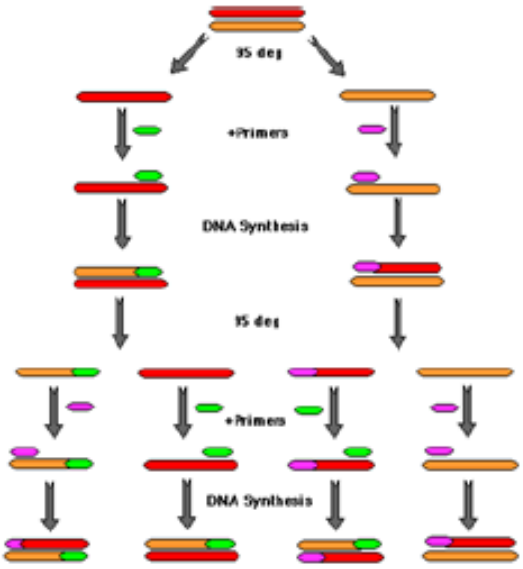

Figure 1. Exponential increase in double-stranded DNA molecules by the polymerase chain reaction (PCR).

Storage/Stability

Store frozen (-20 – 0°C). Stable at least one year when stored as recommended.

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Materials Supplied

N224	**TAQ DNA Polymerase 500U (5U/µL)**
N224-BUF	**TAQ Buffer, 10X (1 mL):** 160 mM (NH4)2SO4, 500 mM Tris-HCl pH 9.2, 17.5 mM MgCl2, 0.1% Triton® X-100
N558-1ML	**PCR Buffer without $MgCl_2$, 10X:** 100 mM Tris-HCl pH 8.3, 500 mM KCl
N560-1ML	**Magnesium Chloride 25 mM Solution**
E636-40UMOLE	**dNTP Mixture, 25 mM Solution (0.4 mL):** 25 mM each dNTP, pH 7.0
E476-1.5ML (x2)	**Water, Sterile, Nuclease-Free**

Required Materials Not Supplied

Forward primer
Reverse primer
DNA template
Thermocycler
PCR reaction tubes or plate
Ice

Protocol/Procedure

PCR reaction setup

Note: Assemble reactions on ice.

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, and Taq polymerase. Consult Table 1 to determine the volume of each component for needed standard 25 µL reactions using the TAQ Buffer, 10X, which

contains $MgCl_2$. Consult Table 2 if using the PCR Buffer without $MgCl_2$, 10X. Prepare at least 20% more master mix than actually needed to account for pipetting losses.

Table 1: Standard 25 µL PCR reaction using TAQ Buffer, 10X containing $MgCl_2$

Reaction Component	Volume
Template DNA	1 – 3 µL (50 – 150 ng)
TAQ Buffer, 10X	2.5 µL
dNTP Mixture, 25 mM Solution	0.2 µL
Forward Primer, 20 µM	0.6 µL
Reverse Primer, 20 µM	0.6 µL
Water, Sterile, Nuclease-Free	As needed to bring final volume to 25 µL
TAQ DNA Polymerase 500U	0.5 µL
Total Volume	**25 µL**

Table 2: 25 µL PCR reaction using PCR Buffer without $MgCl_2$

Note: For larger size DNA products, it is recommended that PCR Buffer without $MgCl_2$ be used and that a titration of the required $MgCl_2$ be performed to determine the optimal reaction conditions. DMSO (not provided) may also be added for reaction enhancement, typically at 5%. Adjust the volumes of the master mix accordingly.

Reaction Component	Volume
Template DNA	1 – 3 µL (50 – 150 ng)
PCR Buffer without $MgCl_2$, 10X	2.5 µL
Magnesium Chloride 25 mM Solution	1.5 µL
dNTP Mixture, 25 mM Solution	0.2 µL
Forward Primer, 20 µM	1 µL
Reverse Primer, 20 µM	1 µL
Water, Sterile, Nuclease-Free	As needed to bring final volume to 25 µL
TAQ DNA Polymerase 500U	0.6 µL
Total Volume	**25 µL**

3. Aliquot the master mix to tubes or wells and add DNA template and primers.
4. Gently vortex the samples and briefly centrifuge to collect the reaction mix in the bottom of the tube or well.

5. Place reactions in a thermocycler and begin PCR. Refer to Table 3 for cycling specifications. The cycling protocol may be adjusted to optimize each sample.

Step	Temperature ºC	Time (Minutes)	Number of Cycles
Initial Denaturation	95	5	1
Denaturation	95	0.25	30
Annealing	50	0.5	
Elongation	69	3	
Final Elongation	69	7	1

Note: During the final elongation step, the terminal tranferase activity of Taq polymerase will incorporate an additional adenosine nucleotide to the 3´ ends of the resulting amplicons. If the PCR fragments are to be TA cloned, the extension step may be extended for up to 30 minutes.

Frequently Asked Questions

Why is there a little yield (or no yield) of PCR product?

- The denaturation step may have been performed at a suboptimal temperature or for an improper length of time.
  - Use an initial denaturation temperature of 95ºC for 3 – 5 minutes, and for the cycling steps use 95ºC for 30 seconds.
- The annealing time or temperature may not have been optimal.
  - Use at least 30 seconds of annealing time to allow primers to bind the template. Select an annealing temperature that is 5ºC lower than the T_m of the lowest T_m primer.
- The extension time may have been insufficient for allowing full target replication.
  - The general rule for selecting extension time is to use 1 minute per each kb of the expected PCR product.
- There may have been an insufficient number of amplification cycles.
  - Replace PCR vials in thermocycler and run an extra 5 cycles. The total number of cycles typically ranges from 20 – 35, depending on the initial template concentration.
- The integrity of the DNA template was compromised or the concentration was too low.

  - Check the DNA quality of the DNA template by agarose gel before using in PCR. Check the concentration of the template by standard methods before use.
- Problems with yield may occur when inhibitors are present in the PCR reaction.
  - Precipitate the original samples or perform a column-based purification. Ions such as EDTA may inhibit the PCR reaction.
- Missing reaction components or improper pipetting may account for low yield.
  - Check the components and set up new reactions.
- Bad primers or primers with incorrect specificity may have been used.
  - Ensure that the 5´ and 3´ ends of primers are not complementary. It is also recommended to avoid a high GC content or extremely mismatched melting temperatures, which may affect binding efficiency. Check the primer sequences to ensure they may bind the template DNA.
- The primer concentration may have been too low or too high.
  - Low primer concentration may decrease annealing efficiency, while excess primers may non-specifically bind to template or to one another. Select a final primer concentration in the range of 0.2 – 1.0 µM.
- The dNTP concentration may have been too low or too high.
  - Use a final concentration of 200 µM for each dNTP in the reaction. Note that using higher concentrations may deplete Mg^{2+}.
- Degraded or low quality dNTPs may have been used.
  - Use the high-quality dNTPs only. Store dNTP solutions frozen in small aliquots to avoid freeze-thaw cycles.
- Mg^{2+} concentration may have been suboptimal.
  - Generally, a final concentration of 1.0 – 6.0 mM Mg^{2+} is recommended. The amount of Mg^{2+} may need to be optimized for each primer pair. Standard reactions typically contain 1.5 mM Mg^{2+}.
- The polymerase concentration may have been too low.
  - The concentration of polymerase may need to be increased depending on the length and difficulty of the template DNA.

Why are there non-specific PCR products?

- The cycling conditions may not have been optimized.
  - Set the thermocycler to no more than 35 cycles total. Choose a lower number of cycles if the initial DNA template is high.
  - The extension time should be set to 1 minute per each kb of expected PCR product.
  - Annealing time and temperature should be 30 seconds and 5ºC lower than T_m of the lowest T_m primer.

    - The ramp speed for the thermocycler is too slow and allows non-specific annealing of primers at temperatures below the set annealing temperature.
- Bad primers or primers with incorrect specificity may have been used.
    - Ensure that the 5´ and 3´ ends of primers are not complementary. It is also recommended to avoid a high GC content or extremely mismatched melting temperatures, which may affect binding efficiency. Check the primer sequences to ensure they may bind the template DNA.
- The primer concentration may have been too high.
    - Excess primers may non-specifically bind to template or to one another. Select a final primer concentration in the range of 0.2 – 1.0 µM.
- An inappropriately high concentration of Mg^{2+} may have been used.
    - Too much Mg^{2+} can increase non-specific product formation. Reduce the Mg^{2+} concentration in the PCR reaction.
- Contamination of the reaction with other templates could result in non-specific product formation.
    - Use dedicated pipettes and filter tips. Set up reactions in a separate location away from where PCR products are handled. Use new reagents if necessary.

Why are there smeared bands when looking at the PCR product by gel?

- Smeared bands may indicate non-specific product formation.
    - See the suggestions above for recommendations.
- Excess template may have been used in the reaction.
    - Too much template may introduce inhibitors that affect polymerase activity in the reaction. Having too much template may also lead to incomplete denaturation. Reduce the template used in the reaction.
- The template may have been sheared of degraded.
    - Prepare a new template. Check the integrity by gel before use.

Directions for Use

For Technical Support

Toll Free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
Email: techinquiry@amresco-inc.com

AMRESCO, LLC
A VWR Company

Corporate Headquarters
28600 Fountain Parkway
Solon, Ohio USA 44139-4300

Tel: 440/349-1199
Fax: 440/349-1182
www.amresco-inc.com

PCR Kit with Taq Polymerase

ZY0649
Rev. 1 12/2015