



## Product Information

### JumpStart™ AccuTaq™ LA DNA Polymerase Mix

Product Code **D 5809**

Technical Bulletin No. MB-1000

## TECHNICAL BULLETIN

### Product Description

JumpStart™ AccuTaq™ LA DNA polymerase mix contains Sigma's high fidelity blend, AccuTaq LA DNA polymerase,<sup>1,2</sup> and the Taq neutralizing JumpStart Taq antibody. This specially formulated enzyme is the best choice for long distance and high fidelity PCR<sup>†</sup>, multiplex PCR and PCR amplification of targets with variable lengths, such as amplification of cDNA libraries. JumpStart AccuTaq LA DNA polymerase mix offers the following unique features:

- Excellent amplification from 0.25 to 22 kb for complex genomic DNA and up to 40 kb for less complex templates.
- Higher fidelity (up to 6.5X) than standard Taq DNA polymerase.
- A hot start mechanism using JumpStart Taq antibody, which prevents non-specific product formation, allows assembled PCR reactions to be placed at room temperature up to 2 hours without compromising performance.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside-triphosphates into acid precipitable DNA in 30 min at 74 °C.

### Reagents

- JumpStart AccuTaq LA DNA Polymerase Mix, Product Code D 5559, 2.5 units/μl AccuTaq LA DNA polymerase and JumpStart Taq antibody in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, 0.5% IGEPAL CA-630, 50% glycerol. Provided as 125, 500, and 1,500 units.
- 10X Buffer for AccuTaq LA DNA Polymerase, Product Code B 0174, 500 mM Tris-HCl, 150 mM ammonium sulfate (pH 9.3, adjusted with NH<sub>4</sub>OH), 25 mM MgCl<sub>2</sub>, 1% Tween 20. Provided as 0.5 ml per vial.

### Storage/Stability

Store JumpStart AccuTaq LA DNA polymerase mix and 10X Buffer at -20 °C.

### Materials and Reagents Required but not Provided

- Deoxynucleotide Mix, Product Code D 7295 (10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM dTTP)
- PCR Reagent Water, Product Code W 1754
- Primers
- DNA to be amplified
- Dedicated pipets
- PCR pipet tips
- 0.2 ml or 0.5 ml thin-walled PCR microcentrifuge tubes, Product Codes P 3114 and P 3364
- Thermal cycler
- Mineral oil, Product Code M 8662 (optional)
- DMSO, Product Code D 8418 (optional)

### Precautions and Disclaimer

Sigma's JumpStart AccuTaq LA DNA polymerase mix is for R&D use only. Not for drug, household or other uses. Refer to the Material Safety Data Sheet (MSDS).

### Preparation Instructions

Reliable amplification of long DNA sequences requires: 1) effective denaturation of DNA template, 2) adequate extension times to produce large products and 3) protection of target DNA from damage by depurination. For best results, optimize the reaction using the following parameters:

### Thermal Cycler

The Perkin-Elmer DNA thermal cyclers 480 and 9700 have been used to develop the cycling parameters. Other types of thermal cyclers can also be used, but may require further optimization of cycling parameters.

### Primer design

Primers are usually 21 to 34 bases long and are designed to have a GC content of 45-50%. Optimally, the melting temperatures of the forward and reverse primers should be within 3 °C of each other and the  $T_M$  of the primers should be between 65 °C and 72 °C.<sup>3,4</sup> Primers should not have any internal base-pairing sequences (i.e., potential hairpins) or complementary regions of any significant length between the two PCR primers.

### Template

An intact, high quality template is essential for reliable amplification of larger fragments. Extreme care must be taken in the preparation and handling of the DNA target for long PCR. Nicked or damaged DNA can serve as a potential priming site resulting in high background. Avoid freezing or, alternatively, freeze only once to minimize damage. Depurination during cycling is minimized by use of buffers with a pH greater than 9.0 at 25 °C. This higher pH limits potential depurination damage to the DNA.

### Magnesium concentration

Optimization of magnesium concentration may be necessary. Generally magnesium concentrations should be between 1 and 5 mM.<sup>5</sup>

### Cycling

Effective denaturation is accomplished by the use of higher temperatures for shorter periods of time. The extension temperature should be limited to 68 °C for optimal performance. Temperatures greater than 68 °C may result in reduced or no product. For targets greater than 20 kb extension times should be greater than 20 minutes. Primer annealing and product extension can also be combined into one step if primers are designed to have a  $T_M$  equal to or greater than 70 °C.

### Buffer preparation

The 10X buffer for AccuTaq LA DNA polymerase mix is at a relatively high pH, and magnesium may precipitate as  $Mg(OH)_2$ . Before use, thaw the buffer at room temperature, then vortex to redissolve any precipitated magnesium hydroxide. Alternatively, warm the buffer at 37 °C for 3-5 minutes, then vortex.

### **Procedure**

The optimal conditions for PCR will depend on the system being utilized. The following protocol serves only as a reference.

1. Add the following reagents to a thin-walled 0.2 ml or 0.5 ml PCR tube:

Volume	Reagent	Final Concentration
5 $\mu$ l	10X Buffer for AccuTaq LA DNA Polymerase	1X
2.5 $\mu$ l	dNTP mix	500 $\mu$ M
--- $\mu$ l	Template DNA	200-500 ng total*
1 $\mu$ l	Forward primer (20 $\mu$ M)	0.4 $\mu$ M
1 $\mu$ l	Reverse primer (20 $\mu$ M)	0.4 $\mu$ M
q.s.	Water	----
1.0 $\mu$ l	JumpStart AccuTaq LA DNA Polymerase Mix (2.5 U/ $\mu$ l)	0.05 units/ $\mu$ l
50 $\mu$ l	Total Volume**	

\*Generally, this is the amount of complex target DNA (such as human genomic DNA) required per reaction. Less DNA is needed for amplification of a simple target, such as lambda DNA.

\*\* The final PCR reaction volume can be scaled down to 20  $\mu$ l by proportionally decreasing each component.

2. Mix gently and briefly centrifuge to collect all components at the bottom of the tube.
3. Add 50  $\mu$ l of mineral oil to the top of each tube to prevent evaporation (optional, depending on model of thermal cycler).
4. Optimum cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximum product yield and/or quality.

### **Typical cycling parameters for a 20 kb genomic DNA fragment**

Initial denaturation	96 °C	30 sec
<b>For cycles 1-30:</b>		
Denaturation	94 °C	5-15 sec
Annealing	62-65 °C	30 sec
Extension	68 °C	20 –25 min
Final extension:	68 °C	30 min
Hold	4 °C	

5. Evaluate the amplified DNA by directly loading 8-10  $\mu$ l of PCR reaction to 0.8–1% agarose gel and subsequent ethidium bromide staining.<sup>6</sup>

**Note:**

When amplifying fragments less than 20 kb, the extension time can be reduced according to the fragment size. Normally, a one-minute extension time will be sufficient for a 1 kb fragment.

**References**

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<sup>†</sup>The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

**JumpStart AccuTaq DNA Polymerase Troubleshooting Guide**

<b>Problem</b>	<b>Possible Cause</b>	<b>Solution</b>
No PCR product is observed	A PCR component is missing or degraded.	A positive control should always be run to insure components are functioning. A checklist is also recommended when assembling reactions.
	Too few cycles were performed.	Increase the number of cycles (3-5 additional cycles at a time).
	The annealing temperature is too high.	Decrease the annealing temperature in 2-4 °C increments.
	The primers are not designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primer to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primer with a GC content of 45-60%.
	There is not enough template.	After increasing the number of cycles has shown no success, repeat the reaction with a higher concentration of template.
	The template is of poor quality.	Evaluate the template integrity by gel electrophoresis. It may be necessary to repurify template using methods that minimize shearing and nicking.
	The denaturation temperature is too high or too low.	Optimize the denaturation temperature by increasing or decreasing the temperature in 1 °C increments.
	The denaturation time is too long or too short.	Optimize the denaturation time by increasing or decreasing it in 10-second increments.
	The extension time is too short.	Increase the extension time in 2-minute increments, especially for long templates.

### JumpStart AccuTaq DNA Polymerase Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
No PCR product is observed (continued)	The reaction does not have enough enzyme.	1.0 $\mu$ l (2.5 units) is sufficient for most applications. It is recommended that the cycling parameters be optimized before the enzyme concentration is increased. In rare cases, the yields can be improved by increasing the enzyme concentration. However, if the enzyme amount is above 2 $\mu$ l (5 units), higher background levels may be seen.
	$Mg^{2+}$ levels are suboptimal.	This is unlikely if the 10X reaction buffer (with $MgCl_2$ ) is used and the deoxynucleotides do not exceed a concentration of 0.6 mM each (as deoxynucleotide triphosphates can bind $Mg^{2+}$ ). Typically, $MgCl_2$ is optimized between 1 to 5 mM. Also, EDTA present in the sample at greater than 5 mM will reduce the effective concentration of magnesium.
	Deoxynucleotide concentration is too low.	This is unlikely if the final concentration of each deoxynucleotide is 0.5 mM. This concentration of dNTPs is suitable for a wide range of applications. If the dNTPs are being prepared in the laboratory, be sure that the final concentration of each deoxynucleotide is 0.5 mM. If the concentration of dNTPs is increased, the $Mg^{2+}$ concentration will need to be increased proportionately.
	Target template is complex.	In most cases, inherently complex targets are due to unusually high GC content and/or secondary structure.
There are multiple or smeared products	The annealing temperature is too low.	Increase the annealing temperature in increments of 2-3 $^{\circ}$ C.
	The primers are not designed optimally.	Confirm the accuracy of the sequence information. If the primers are less than 27 nucleotides long, try to lengthen the primers to 27-33 nucleotides. If the primer has a GC content of less than 45%, try to redesign the primers with a GC content of 45-60%.
	Touchdown PCR may be required.	"Touchdown" PCR significantly improves the specificity of many PCR reactions in various applications. Touchdown PCR involves using an annealing/extension temperature that is higher than the $T_M$ of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer $T_M$ for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles. <sup>7</sup>
	Too many cycles were performed.	The nonspecific bands may be eliminated by reducing the number of cycles.
	There is too much enzyme in the reaction mix.	1 $\mu$ l (2.5 units) is sufficient for most applications. However, this concentration may be too high for some applications. We recommend optimizing the cycling parameters first as described above, then if necessary incrementally reduce the enzyme concentration to determine the optimal concentration.

### JumpStart AccuTaq DNA Polymerase Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
There are multiple or smeared products (continued)	Magnesium concentration is too high.	The MgCl <sub>2</sub> concentration should be optimized. Typically, the concentration of MgCl <sub>2</sub> is optimal between 1 and 5 mM. If the concentration of the dNTPs is 0.5 mM, it is very unlikely that the magnesium concentration is too high.
	The template concentration is too high.	Reduce the concentration of the template in the PCR reaction.
	The template concentration is too low.	Add additional template in 50 ng increments for genomic DNA or 1-2 ng for viral DNA.
There is no reduction of nonspecific PCR bands when using the JumpStart enzyme.	The antibody affinity may be reduced by reaction components or conditions.	Some cosolvents, solutes (e.g., salts) and pH extremes may reduce the affinity of the JumpStart Taq antibody for the polymerase and thereby compromise its effectiveness. Check your reaction mixture and conditions and/or check your system with a manual hot start method.
	Primers were not designed appropriately.	Check your system with a manual hot start method. If the results are similar, raise the annealing temperature in 2-3 °C increments to improve the specificity of binding. If raising the temperature reduces the yield of the specific product with only a small reduction of side reaction products, it may be necessary to redesign the primers. <sup>8</sup>
	There was crossover contamination of specific and/or nonspecific PCR products.	Take special precautions to avoid crossover contamination of PCR reactions, including primer-dimer artifacts. <sup>9</sup>
The yield of specific product is low.	Too few cycles were performed.	Increase the cycle number in 3-5 cycle increments.
	Extension times are too short.	Increase the extension times in 2-minute increments.
	A co-solvent is required.	Add dimethyl sulfoxide up to a final concentration of 5%.
	PCR priming opportunities may be low due to reaction conditions or primer design.	Modify the reaction conditions by increasing the denaturation temperature to 95 °C, increase extension times in 2-minute increments, increase MgCl <sub>2</sub> and dNTP concentrations, etc. Redesign PCR primers.

## Related Products

### Reagents and Kits

- PCR Optimization Kit, Product Code OPT-2
- Lambda Hind III DNA Marker, Product Code D 9780
- BlueView™ Nucleic Acid Stain, Product Codes T 8935 and T 9060. When added to agarose gel and running buffer, BlueView instantly stains nucleic acids.
- Enhanced Avian HS RT-PCR kits, Product Codes HSRT-20 (20 reactions) and HSRT-100 (100 reactions). The RT-PCR kit combines two powerful techniques to convert mRNA into cDNA and subsequently to amplify the cDNA. eAMV-RT has an enhanced ability to transcribe through complex secondary structure at elevated temperatures (up to 65 °C). Includes JumpStart AccuTaq LA DNA polymerase mix for hot start PCR.

### Techware

- PCR Multiwell Plates, 96-well, Product Code Z37,490-3
- PCR Multiwell Plates, 384-well, Product Code Z37,491-1
- PCR Microtubes, 0.2 ml with attached caps, Product Code Z37,487-3
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Product Code Z37,496-2
- Sealing accessory for PCR vessels, Pierceable cap strips, Product Code Z37,495-4, caps in strips of eight, the center of each can be pierced with a hypodermic needle for quick sample removal without generating aerosols or other sources of cross-contamination. Caps can be used with 0.2 ml PCR strip and 96-well plates. Pkg of 120 strips (960 caps).
- Sealing accessory for PCR vessels, Micro Mats, Product Code Z37,493-8, molded to fit standard 96-well plates
- PCR Workstation, 120V, Product Code Z37,621-3
- PCR Workstation, 240V, Product Code Z37,622-1

### PCR Books

- *PCR: Essential Data Series*, C.R. Newton, Ed., John Wiley and Sons, Inc., New York, NY, 1995, Product Code Z36,491-6
- *PCR: A Practical Approach*, M.J. McPherson, P. Quirke and G.R. Taylor, Eds., IRL Press, Oxford, England, 1991, Product Code P 7186
- *PCR 3: PCR In Situ Hybridization*, C.S. Herrington and J.J. O'Leary, Eds., IRL Press at Oxford University Press, Inc., Oxford, England, 1997, Product Code Z37,839-9
- *PCR Cloning Protocols: Methods in Molecular Biology*, Vol. 67, B. White, Ed., Humana Press, Totowa, NJ, 1996, Product Code Z37,422-9
- *PCR In Bioanalysis: Methods in Molecular Biology*, Vol. 92, S.J. Smeltzer, Ed., Humana Press, Totowa, NJ, 1998, Product Code Z37,960-3
- *PCR Primer: A Laboratory Manual*, C. Dieffenbach and G.S. Dvekster, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1995, Product Code Z36,411-8
- *PCR Protocols: A Guide to Methods and Applications*, M.A. Innis, et al., Eds., Academic Press, San Diego, CA, 1990, Product Code P 8177
- *PCR Protocols for Emerging Infectious Diseases*, D.H. Persing, Ed., American Society for Microbiology, Washington, DC, 1996, Product Code Z36,991-8
- *PCR Sequencing Protocols*, R. Rapley, Humana Press, Totowa, NJ, 1996, Product Code Z37,381-8
- *PCR Strategies*, Michael A. Innis, David H. Gelfand, and John J. Sninsky, Eds., Academic Press, San Diego, CA, 1995, Product Code Z36,445-2
- *PCR Technology, Current Innovations*, H.G. Griffin and A.M. Griffin, Ed., CRC Press, Boca Raton, FL, 1994, Product Code Z35,749-9
- *Quantitation of mRNA by Polymerase Chain Reaction*, T. Kohler, et al., Springer-Verlag, Berlin, 1995, Product Code Z37,194-7

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