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Product Information

JumpStart™ REDAccuTaq® LA DNA Polymerase

Catalog Number **D1313**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

JumpStart™ REDAccuTaq® LA DNA Polymerase contains AccuTaq LA DNA polymerase,[1,2] an inert red dye, and JumpStart *Taq* antibody. This specially formulated enzyme offers several unique features:

- The enzyme of choice for long distance and high fidelity PCR, multiplex PCR, and PCR amplification of targets with variable lengths, such as amplification of cDNA libraries.
- 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 6.5×) 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.
- The red tracer provides quick recognition of reactions to which the enzyme has been added as well as visual confirmation of complete mixing.
- The enzyme is provided at one unit/µL for more accurate volume measurement and less waste.
- The enzyme formulation allows aliquots (5-10 µL) from the PCR to be directly loaded onto an agarose gel without addition of loading buffers.
- The red tracer serves as a tracking dye co-migrating at the same rate as a 125 bp fragment in a 1% agarose gel.

Since the red tracer has no effect on the amplification process, a sample can be easily re-amplified as in "nested PCR". The presence of the dye also has no effect on automated DNA sequencing, ligation, exonucleolytic PCR product digestion, and transformation. Although exceptions may exist, the dye is generally inert in restriction enzyme digestions. If necessary, the dye can be removed from the amplicon by routine purification methods.

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

Reagents

- JumpStart REDAccuTaq LA DNA Polymerase, Catalog Number D1938 – 1 unit/µL AccuTaq LA DNA polymerase with JumpStart Taq antibody in 20 mM Tris-HCl, pH 8.0, 20 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% TWEEN® 20, 0.5% IGEPAL® CA-630, 50% glycerol, inert dye. Provided as 50, 250, and 2500 units.
- AccuTaq LA 10× Buffer, Catalog Number B0174, 0.5 ml vial,
  500 mM Tris-HCl, 150 mM ammonium sulfate (pH 9.3, adjusted with NH_4OH), 25 mM $MgCl_2$, 1% TWEEN 20). Provided as 3 vials/50 units and 3 vials/250 units.

Materials and reagents required but not provided

- Deoxynucleotide Mix, Catalog Number D7295 10 mM dATP, dCTP, dGTP, TTP
- Water, PCR Reagent, Catalog Number W1754
- Primers
- DNA to be amplified
- Dedicated pipets
- PCR pipet tips
- 0.2 ml or 0.5 ml thin-walled PCR microcentrifuge tubes, Catalog Numbers P3114 and P3364
- Thermal cycler
- Mineral oil, Catalog Number M8662 (optional)
- Betaine solution, 5 M, Catalog Number B0300 (optional)
- DMSO, Catalog Number D8418 (optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store JumpStart REDAccuTaq LA DNA Polymerase and 10× Buffer at –20 °C.

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 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 45-60%. 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 65-72 °C.[3,4] 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 DNA.

Magnesium concentration
Optimization of magnesium concentration may be necessary. Generally magnesium concentrations should be between 1 and 5 mM.[5]

Cycling
Effective denaturation is accomplished by using 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 AccuTaq LA 10X Buffer is at a relatively high pH, and magnesium may precipitate as magnesium hydroxide [$Mg(OH)_2$]. Before use, thaw the buffer at room temperature, then vortex to redissolve any precipitated $Mg(OH)_2$. 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 µL	AccuTaq LA 10× Buffer	1×
1.5 µL	dNTP mix	300 µM*
- µL	Template DNA	200-500 ng total**
1 µL	Forward primer (20 µM)	0.4 µM
1 µL	Reverse primer (20 µM)	0.4 µM
q.s.	Water, PCR Reagent	----
2.5 µL	JumpStart REDAccuTaq LA DNA Polymerase (1 unit/µL)	0.05 units/µL
50 µL	Total Volume***	

* dNTP concentration can range from 300-500µM. However, higher dNTP concentrations can inhibit amplification for some applications.
**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 µL by proportionally decreasing each component.

2. Mix gently and briefly centrifuge to collect all components at the bottom of the tube.

3. Add 50 µL 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.

Troubleshooting Guide

Typical cycling parameters for a 20 kb genomic DNA fragment

Initial denaturation	96 °C	30 sec
For cycle 1-30:		
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 µL of PCR reaction to 0.8 –1% agarose gel and subsequent ethidium bromide staining.[6]

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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10. Kwok, S. and Higuchi, R., *Nature*, **339**:237-238 (1989).

Problem	Possible Cause	Solution
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.
	The reaction does not have enough enzyme.	0.05 units/µl 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 concentration is above 0.10 units/µL, higher background levels may be seen.
	Mg^{2+} levels are suboptimal	This is unlikely if the 10× 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 0.3 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 0.3 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. Betaine has been reported to help amplification of high GC content templates at a concentration of 0.8-1.3 M.[7]

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
There are multiple or smeared products	The annealing temperature is too low.	Increase the annealing temperature in increments of 2-3 °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 made in a single step or in increments over several cycles.[8]
	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.	0.05 units/µL is sufficient for most applications. However, this concentration may be too high for some applications. It is recommended the cycling parameters be optimized first, as described above, then reduce the enzyme concentration to 0.5-0.2X.
	Magnesium concentration is too high.	The $MgCl_2$ concentration should be optimized. Typically, the concentration of $MgCl_2$ is optimal between 1 and 5 mM. If the concentration of the dNTPs is 0.3 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 co-solvents, 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.[9]
	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.[10]

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
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 (5%) or 0.8-1.3 M betaine final concentration.
	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 $MgCl_2$ and dNTP concentrations, etc. Redesign the PCR primers.
The finished PCR reaction does not sink in the well of the agarose gel	There is too little REDAccuTaq in the reaction mix; the mix was diluted.	Add loading buffer to the reaction aliquot.

Related Products

Reagents

- Lambda DNA Hind III Digest, Catalog Number D9780
- Enhanced Avian HS RT-PCR kit, Catalog Number HSRT100 (100 reactions). This kit combines enhanced avian reverse transcriptase and JumpStart AccuTaq LA for hot start PCR.
- BlueView™ Nucleic Acid Stain, Catalog Numbers T8935 and T9060.

Equipment

- PCR Multiwell Plates, 96-well, Catalog Number Z374903; 384-well, Catalog Number Z374911
- PCR Microtubes, 0.2 ml with attached caps, Catalog Number Z374873; 0.2 ml strip tubes with strip caps, Catalog Number Z374962
- Micro Mats, Catalog Number Z374938, molded to fit standard 96-well plates
- PCR Workstation, 120V, Catalog Number Z376213; 240V, Catalog Number Z376221

Books

- *PCR Primer: A Laboratory Manual, 2nd ed.,* Catalog Number Z701270

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