User Guide

JumpStart™ *Taq* DNA Polymerase

With 10X reaction buffer containing 15mM $MgCl_2$

D9307

Product Overview

Description

JumpStart™ *Taq* DNA Polymerase is an optimized combination of high-performance *Taq* DNA Polymerase, JumpStart™ *Taq* antibody, and an inert red dye tracer. The neutralizing monoclonal antibody binds to *Taq* DNA polymerase, inactivating it until the complex is dissociated at ≥70 °C, providing simple and efficient hot start PCR. Because JumpStart™ *Taq* DNA polymerase is inactive at room temperature, reaction mixtures can be prepared on the bench and transported directly to the thermocycler as the enzyme will become active in the first denaturation step of the cycling process.

The enzyme is supplied at 2.5 units/µL and comes with an optimized 10X reaction buffer containing 15 mM $MgCl_2$.

Features

- Reduces nonspecific amplification and primer-dimers
- Performs equivalent to, or better than, standard *Taq* polymerase
- Allows for room-temperature PCR set up
- Suitable for large number of thermal cycles (>35)
- Amplified product length up to 3 kb

Applications

JumpStart™ *Taq* can be used for amplification of:

- Genomic DNA
- cDNA
- Low copy number targets
- Multiple targets (multiplex PCR)

Unit Definition

One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

Reagents Provided

- JumpStart™ *Taq* DNA polymerase, 2.5 units/µL in 50% glycerol, 87.5 mM KCl, 17.5 mM Tris-HCl, pH 8.0, 75 µM EDTA, 7.5 mM DTT, and stabilizers (D6558)
- 10X PCR Buffer, 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatin (P2192)

Materials and Reagents Required

(But not included)

- Deoxynucleotide (dNTP) Mix, containing 10 mM each of dATP, dCTP, dGTP, and dTTP sodium salts
- Nuclease-free water
- Custom ordered primers specific to gene target
- PCR tubes or plates
- Sample containing template DNA
- Thermal cycler

Precautions and Disclaimer

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

Storage/Stability

Store at –20 °C. Storing JumpStart™ *Taq* below –20 °C is not recommended.

Procedure for Use

Step	Description

Assemble Reaction Mix

For best reproducibility, assemble a master mix of PCR reagents by multiplying the number of reactions needed (plus 10% to account for pipetting error) by the suggested volumes in the table below. Aliquot reaction mixture into PCR tubes.

Reagent	Final Concentration	Amount per 20 µL reaction
10X PCR buffer	1X	2 µL
JumpStart™ *Taq*	0.05 U/µL	0.4 µL
dNTP Mix, 10 mM	200 µM	0.4 µL
Primers	0.1–0.5 µM	Variable
Template	Variable	Variable
Nuclease-free Water	-	To 20 µL total

Note: JumpStart™ *Taq* is a magnesium ion-dependent enzyme, optimal concentrations of template DNA, primers, and $MgCl_2$ will be target-specific. The supplied 10X PCR buffer contains 15 mM $MgCl_2$, for a final concentration of 1.5 mM; however, the final $MgCl_2$ concentration can be adjusted up to 3.5 mM for endpoint assays, if necessary.

Add Template

Recommended input template is 10 ng DNA; however, JumpStart™ *Taq* may amplify as little as a single copy of non-complex template or 10-100 copies of complex genomic template. For cDNA templates use a 1:10 reaction dilution for medium to highly expressed targets, or a 1:2 to 1:5 dilution for low expression targets.

Amplify

A suggested thermocycling protocol using JumpStart™ *Taq* is provided below:

Initial denaturation		94 °C	2 min
40 cycles	Denaturation	94 °C	15 sec
	Annealing	60 °C or 5 °C below lowest primer T_m	30 sec
	Extension	72 °C	1 min/kb
Final extension		72 °C	1 min
Hold		4 °C	∞

Amplification parameters will vary depending on primers, template, and instrument used. For tips on optimizing PCR conditions as well as a 2-step cycling protocol please see Technical Guide.

Evaluate

Amplified DNA can be evaluated by any standard method, including agarose gel electrophoresis, fluorescent dye intercalation, and DNA sequencing.

Technical Guide

Considerations for Primer Design

Thoughtful primer design is essential for PCR efficiency and specificity. For successful amplification consider the following:[1]

- Select an 18-30 nucleotide-long sequence with 40-60% G/C content and even distribution of all 4 bases.
- Avoid inverted repeat or self-complementary sequences >3 bp.
- Primer pairs should not differ in length by >3 bp and should not contain complementarity to one another.
- Maintain calculated primer T_m between 55-60 °C, permitting only 2-3 °C variation between primer pairs.
- Priming efficiency can be increased by including a terminal G at the 3' end; however, the number of Gs or Cs in the last 5 bases of the primer sequence should be no more than 3.
- Ensure each primer sequence is unique to the gene of interest and is absent in other genes in the gDNA sample or within the vector.

Optimization of PCR Conditions

PCR involves the cycling of denaturing, annealing, and extension steps for DNA synthesis by a polymerase enzyme. To obtain the best product yield and accuracy, each step must be optimized.

- The **denaturing** step (94-96 °C) activates the JumpStart™ *Taq* DNA polymerase and separates double-stranded DNA strands, making it accessible to primers.[1] The duration of this step should be long enough to denature DNA but not so long that it compromises *Taq* DNA polymerase integrity.[1] High salt conditions, GC-rich (>55%) templates, and gDNA templates may require longer denaturation times and/or higher temperatures. For maximum retention of JumpStart™ *Taq* activity during thermocycling, use 94 °C for denaturation.
- The **annealing** temperature can be calculated by subtracting 5 °C from the lowest reaction primer T_m. The annealing time should be long enough for the primer to anneal to the template but not too long for non-specific annealing to occur.[1]
- The optimal **extension** temperature for *Taq* DNA polymerase is 72 °C; however, lower temperatures may be used for some reactions. Extension time depends on length and complexity of the target sequence. For complex templates, use 1 minute/kb, with 15 seconds added if the PCR product is >2 kb. Short or non-complex templates may be amplified with extension times of 30 seconds/kb.
- The **number of cycles** needed for amplification depends on the amount of template input, with higher amount of input requiring less cycling.[1] Generally, 25-30 cycles are sufficient to produce detectable product; however, low concentration templates may require up to 45 cycles.
- To maintain **enzyme fidelity**, or accuracy of nucleotide incorporation, limit the number of PCR cycles and use an equimolar concentration of each dNTP.[1] Magnesium ion concentration also affects enzyme fidelity at a concentration that varies by reaction conditions and should thus be optimized.[1]

Handling gDNA Templates

To prevent genomic DNA (gDNA) shearing, add template last and mix gently using a wide pore pipet tip. DO NOT VORTEX!

Multiplex PCR

When performing multiplex PCR, competition between products for reagents may occur. Consider adjusting the following for optimization:[2]

- Proportion of primer pair concentration: if a target sequence produces a relatively "weaker" signal, the amount of primer used may be increased to compensate. For sequences with low copy numbers, or high-complexity, primer concentration can be used at 0.3-0.5 μM.

  Primer concentration can also be decreased for target sequences producing "stronger" signal to achieve balance. For high copy number or low-complexity sequences, primer concentration can be used at 0.04-0.4 μM.

- dNTP: Perform a stepwise increase of dNTP to a concentration ≤400 μM. Keep $MgCl_2$ concentration constant for this optimization.

- PCR Buffer: Use 2X buffer concentration for the reaction instead of 1X.

- Supplement the reaction with additional $MgCl_2$, or PCR-enhancing additives.

PCR-Enhancing Additives

When optimizing PCR conditions for a new experiment, the following can be added to the reaction mix individually. After performing PCR amplification, samples with and without additive can be compared using agarose gel electrophoresis or other standard methods to look for improved product specificity and yield.

Additive	Purpose
Bovine Serum Albumin (10-100 µg/mL)	*Taq* DNA polymerase stabilization[3]
Formamide (1.25-10%)	Increases specificity in G/C rich regions[4]
DMSO (Up to 5%)	Accelerates strand renaturation[5] Nucleic acid thermal stability against depurination[5]
Glycerol (Up to 10%)	Increases thermal stability of the polymerase and lowers the temperature necessary for strand separation[5]
Ammonium sulfate (15-30 mM)	Affects the denaturing and annealing temperatures of the DNA[6]
Single strand binding protein (0.7-1.5 µg)	Inhibits formation of secondary structures, improving fidelity and *Taq* processivity[7]
Betaine (0.8-1.6 M)	Reduces base pair composition dependence of DNA melting[8]

Two-Step PCR Amplification

Application of a two-step PCR process is possible when the annealing and extension temperatures are similar.

	Initial denaturation	94 °C	2 min
40 Cycles	Denaturation	94 °C	3 sec
	Annealing/extension	60 °C*	15-30 sec
	Final extension**	72 °C	1 min
	Hold	4 °C	∞

*Consult primer T_m regarding temperature selection. Extension time is target-dependent, with larger targets requiring more than the recommended time.

**The final extension step is needed when the objective is to use the PCR product for downstream applications (e.g. cloning, or agarose gel electrophoresis), and is otherwise optional (e.g. for qPCR).

Product Ordering

Description	Catalogue Number
JumpStart™ *Taq* DNA Polymerase	D4184
Magnesium chloride solution	M8787
Deoxynucleotide (dNTP) Mix, containing 10 mM each of dATP, dCTP, dGTP, and dTTP sodium salts	D7295
Nuclease-free water	W1754
Custom ordered primers specific to gene target	OLIGO
GenElute™-E Single Spin DNA Cleanup Kit	EC600
GenElute™ Bacterial Genomic DNA Kit	NA2120
GenElute™ Mammalian Genomic DNA Miniprep Kit	G1N10
GenElute™ Plant Genomic DNA Miniprep Kit	G2N70
GenElute™ PCR Clean-Up Kit	NA1020
GenElute™ Gel Extraction Kit	NA1111
Precast Agarose Gels	P6222 P5472 P6097 P5972 P5722
1 kb DNA Ladder	D0428
Water, Microbial DNA-free	MBD0025
Nuclease-Free Water, for Molecular Biology	W4502
JumpStart™ *Taq* Ready Mix	P2893
JumpStart™ RED*Taq*® Ready Mix	P0982
Glycerol-free JumpStart™ *Taq* DNA Polymerase	D9310
DMSO	D8418
Single strand binding protein	S3917
Betaine solution	B0300
Mineral Oil	M5904
Bovine Serum Albumin solution	B8667

Troubleshooting Guide

Problem	Suggestions
No or low product amplification	• Titrate $MgCl_2$ concentration in 0.5 mM increments using molecular biology grade $MgCl_2$ solution (not provided, see Product Ordering). The recommended range for endpoint PCR assays is 1.5-3.5 mM $MgCl_2$. Each amplicon target must be optimized individually. • Adjust the annealing temperature in 2-3 °C increments or use a gradient PCR to find the optimal annealing temperature. • Increase the number of amplification cycles. If currently using 25-30 cycles, increase the cycle number to 35-40. • For complex templates like human genomic DNA, increase the initial denaturation time by 1-2 minutes and/or increase the denaturation temperature to 95 °C to overcome denaturation difficulties. • Check concentration of input template. For complex templates like intact eukaryotic genomic DNA, 1000 genome copies may be required for amplification of difficult targets. For highly concentrated templates, such as purified plasmid, consider diluting 1:1000 to improve amplification. • Assess DNA quality to ensure absence of PCR inhibitors in sample. If presence of inhibitors is suspected, DNA can be diluted 1:10-1:100. Alternatively, lysis and DNA purification can be performed using the GenElute™ genomic DNA miniprep kits. • Refer to "PCR-Enhancing Additives" section of the Technical Guide (page 3-4) to improve amplification. • If yield is too low for downstream applications, increase the reaction volume to 50-75 µL.
Amplification of nonspecific product(s)	• Raise the annealing temperature in 2-3 °C increments or use a gradient PCR to find the optimal annealing temperature. Raising the temperature improves the specificity of binding by the primers; however, it may also result in reduced binding and extension of the primers.[1] If raising the annealing temperature causes reduced yield of the specific product without eliminating side reaction products, it may be necessary to redesign the primers to improve specificity. • Take precautions to avoid crossover contamination of PCR with both specific and nonspecific PCR products, including primer-dimer artifacts.[9] • Titration of JumpStart™ *Taq* may be necessary to optimize PCR efficiency, especially if the reaction conditions vary from those recommended in this document. In this case, increase the concentration of JumpStart™ *Taq* by two- or four-fold. Increasing the concentration of JumpStart™ *Taq* beyond this level may inhibit PCR. • The use of more than 5% v/v DMSO with JumpStart™ *Taq* is not recommended as it may interfere with the enzyme-antibody complex. Other co-solvents, salts, and extremes in pH can also reduce the affinity of the JumpStart™ *Taq* antibody for the *Taq* DNA Polymerase and compromise its effectiveness for hot start PCR.

References

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