

# HOT START PCR

## Hot Start PCR Selection Guide

Catalog Number	Product Description	Fidelity Compared to Standard Taq	Amplification Length <sup>a</sup>	Direct Loading <sup>b</sup>	Easy MgCl <sub>2</sub> Optimization <sup>c</sup>	Assembled Master Mix <sup>d</sup>	Page
D9307	JumpStart Taq DNA Polymerase	1×	0.1 to >3 (10) kb				11
D4184	JumpStart Taq DNA Polymerase without MgCl <sub>2</sub>	1×	0.1 to >3 (10) kb		✓		11
D8187	JumpStart REDTaq DNA Polymerase	1×	0.1 to >3 (10) kb	✓			12
P2893	JumpStart Taq ReadyMix	1×	0.1 to >3 (10) kb			✓	13
P0982	JumpStart REDTaq ReadyMix PCR Reaction Mix	1×	0.1 to >3 (10) kb	✓		✓	13
P1107	JumpStart REDTaq ReadyMix For High Throughput PCR	1×	0.1 to >3 (10) kb	✓		✓	13
D5809	JumpStart AccuTaq LA DNA Polymerase	up to 6.5×	0.1 to >20 (40) kb				14
D1313	JumpStart REDAccuTaq LA DNA Polymerase Mix	up to 6.5×	0.1 to >20 (40) kb	✓			14
D0816	JumpStart KlenTaq LA DNA Polymerase	up to 4×	0.1 to >5 (20) kb				15
A7721	JumpStart Taq Antibody						16

- a) Two values are provided for the indicated amplification length. The range provided refers to the average length achieved from complex genomic targets, while the number in parentheses refers to lengths routinely achieved with less complicated targets such as plasmid or lambda phage DNA.
- b) REDTaq and REDAccuTaq products contain an inert red dye. The red dye confirms the polymerase has been added to the reaction and complete mixing has occurred. Aliquots from the PCR can be directly loaded onto the gel without the need for loading buffers or tracking dyes. The dye does not interfere with automated DNA sequencing, ligation, transformation or other downstream applications.
- c) The 10× PCR Buffer does not include MgCl<sub>2</sub>. A separate vial of 25 mM MgCl<sub>2</sub> is included to permit optimization.
- d) Each ReadyMix is conveniently supplied at 2× concentration and prepared using the indicated thermostable DNA polymerase, ultrapure 99%+ dNTPs and high quality molecular biology reagents. To prepare a 50 µl PCR reaction, simply add 25 µl of the appropriate ReadyMix to 25 µl of water containing primers and template.

## JumpStart™ Taq DNA Polymerase

### Increase Specificity and Yields

Sigma's JumpStart Taq DNA Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield. Once the reaction temperature reaches 70 °C, Taq DNA Polymerase activity is restored and the resulting PCR exhibits a higher specificity and yield. This antibody-enzyme complex allows for easy and convenient set-up with less contamination risk than manual hot start techniques. Since the enzyme can be included in the master mix preparation, more consistent results are obtained from one reaction to the next.

### Features and Benefits

- Minimize non-specific amplification while increasing target yield and specificity
- Superior amplification regardless of template concentration
- Reduce set-up time and eliminate concerns associated with manual or wax hot start methods

JumpStart Taq DNA Polymerase is provided with a 10× Reaction Buffer available with and without MgCl<sub>2</sub>. The 10× Buffer without MgCl<sub>2</sub> includes a separate tube of 25 mM MgCl<sub>2</sub> for optimization.

**Components:** JumpStart Taq DNA Polymerase 10× PCR Buffer or 10× PCR Buffer without MgCl<sub>2</sub> and a separate tube of 25 mM MgCl<sub>2</sub>

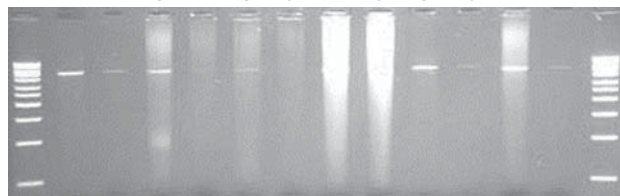
**Unit definition:** One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

**Concentration:** 2.5 units per µl

**Storage:** -20 °C  
Shipped in wet ice

**Inactivated by a Taq-Directed Antibody, JumpStart Taq Provides Increased Sensitivity, Specificity, and Yield**

Sigma    Comp. A    Comp. Q    Comp. R    Comp. C    Comp. I  
1    2    3    4    5    6    7    8    9    10    11    12



Ten nanograms (even lanes) or 100 nanograms (odd lanes) total human genomic DNA was amplified with primers targeted to a 5 kb section of  $\beta$ -globin. Each PCR was performed according to supplier's recommendations.

Cat. No.	Product Description	Quantity
D9307	JumpStart Taq DNA Polymerase	50 units 250 units 1,500 units
D4184	JumpStart Taq DNA Polymerase without MgCl <sub>2</sub>	50 units 250 units 1,500 units

# HOT START PCR

## JumpStart™ REDTaq® DNA Polymerase

JumpStart REDTaq DNA Polymerase is a specialized blend of REDTaq Genomic DNA Polymerase and JumpStart Taq Polymerase. JumpStart Taq Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield. Unlike other hot start methods (i.e. chemical inactivation), JumpStart Taq Polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction.

JumpStart REDTaq DNA Polymerase has the benefits of REDTaq Genomic DNA Polymerase including enhanced amplification of genomic templates, easy visualization of enzyme addition, complete reaction mixing, and direct loading of samples following amplification.

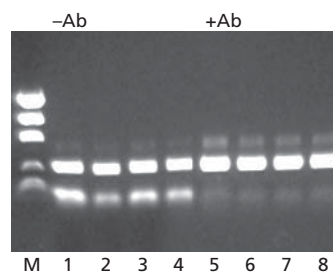
The inert red dye does not affect automated sequencing, restriction enzyme digestion, ligation or other downstream applications. The PCR product can be easily separated from the dye by standard purification methods.

### Features and Benefits

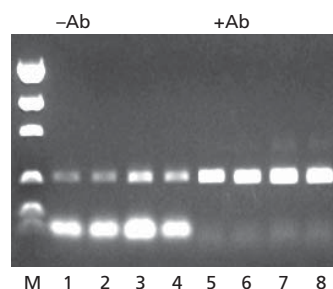
- Minimize non-specific amplification while increasing target yield and specificity
- Superior amplification regardless of template concentration
- Reduce set-up time and eliminate concerns associated with manual or wax hot start methods. JumpStart Taq minimizes non-specific amplification while increasing target yield. The red dye also provides quick recognition and confirmation of appropriate mixing. Aliquots can be loaded directly on an agarose gel without the need for loading buffers or tracking dyes

The enzyme is provided with an optimized 10× Reaction Buffer.

### Superior Amplification Over a Wide-Dynamic Range of Template



**Figure 1.** M = DNA Marker; Lanes 1-4: Standard PCR on 4 ng template DNA; Lanes 5-8: Hot Start PCR using JumpStart REDTaq on 4 ng template DNA



**Figure 2.** M = DNA Marker; Lanes 1-4: Standard PCR on 0.4 ng template DNA; Lanes 5-8: Hot Start PCR using JumpStart REDTaq on 0.4 ng template DNA.

**Components:** JumpStart Taq DNA Polymerase  
10× PCR Buffer

**Unit definition:** One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

**Concentration:** 1 unit per  $\mu$ l

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
D8187	JumpStart REDTaq DNA Polymerase	50 units 250 units 2,500 (10 x 250) units

# HOT START PCR

## JumpStart™ Taq ReadyMix™ PCR Reaction Mixes

JumpStart Taq ReadyMix PCR Reaction Mix is a prepared solution combining the performance benefits of hot start PCR with the convenience of a ReadyMix. The mix includes Sigma's high quality JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and buffer in a 2× optimized reaction concentrate. Add 25 µl of ReadyMix to template, primers and water to a final reaction volume of 50 µl. JumpStart Taq Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield. Unlike other hot start methods (i.e. chemical inactivation), JumpStart Taq polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction.

Using JumpStart Taq ReadyMix reduces pipetting steps and risk of contamination. The hot start mechanism allows for room temperature set-up, making this the product of choice for high throughput applications.

### Features and Benefits

- Minimize non-specific amplification while increasing target yield and specificity
- Superior amplification regardless of template concentration
- Reduce set-up time and eliminate concerns associated with manual or wax hot start methods. JumpStart Taq minimizes non-specific amplification while increasing target yield

### Available in Direct Load

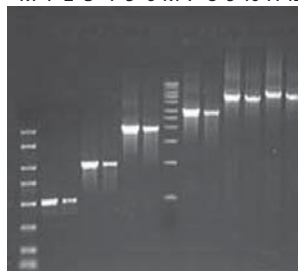
JumpStart REDTaq ReadyMix PCR Reaction Mix combines the advantages of JumpStart Taq ReadyMix with the added convenience of an inert red dye. This dye provides quick visual confirmation that the enzyme has been added and properly mixed. After PCR, an aliquot can be loaded directly onto an agarose gel without the need for loading buffers or tracking dyes.

## Available in a High Throughput, Genomic Formulation

JumpStart REDTaq ReadyMix PCR Reaction Mix for High Throughput PCR is formulated with REDTaq Genomic DNA Polymerase for amplification of more complex or genomic templates. This mix contains optimized enzyme and dye concentrations to provide increased length and yield on more difficult templates.

### Exceptional Performance with JumpStart REDTaq ReadyMix Hot Start PCR in the Convenience of a ReadyMix

M 1 2 3 4 5 6 M 7 8 9 10 11 12



200 ng Lambda phage DNA was amplified with Sigma's JumpStart REDTaq ReadyMix (odd numbered lanes) and Competitor's Direct Load ReadyMix (even numbered lanes). Taq was activated per the supplier's recommendations.

**Unit definition:** One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
P2893	JumpStart Taq ReadyMix 1.5 units Taq/reaction (50 µl reaction volume)	100 reactions 400 reactions
P0982	JumpStart REDTaq ReadyMix PCR Reaction Mix 1.5 units Taq/reaction (50 µl reaction volume)	20 reactions 100 reactions
P1107	JumpStart REDTaq ReadyMix PCR Reaction Mix for High Throughput PCR 0.75 units Taq/reaction (50 µl reaction volume)	100 reactions 400 reactions

# HOT START PCR

## JumpStart™ AccuTaq™ LA DNA Polymerase

### Increase Yields, Fidelity and Specificity

JumpStart AccuTaq LA DNA Polymerase is a combination of AccuTaq LA DNA Polymerase plus a Taq-directed antibody. JumpStart Taq Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield. Unlike other hot start methods (i.e. chemical inactivation), JumpStart Taq Polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction. JumpStart AccuTaq LA DNA Polymerase can generate long products with higher fidelity (up to 6.5× of Taq DNA Polymerase).

### Features and Benefits

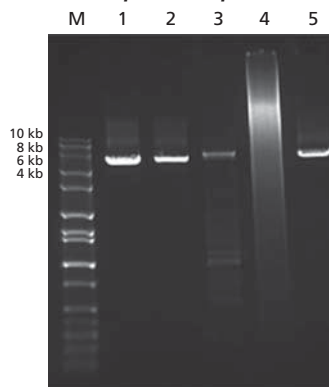
- Minimize non-specific amplification while increasing target yield and specificity
- Fidelity up to 6.5× that of Taq DNA Polymerase making it the enzyme of choice for multiplex PCR
- Produce amplicons up to 22 kb on genomic templates and up to 40 kb on less complex templates such as lambda or bacterial genomic DNA
- Reduce set-up time and eliminate concerns associated with manual or wax hot start methods. JumpStart Taq minimizes non-specific amplification while increasing target yield

Supplied with 10× Reaction Buffer.

### Available in Direct Load

JumpStart REDAccuTaq LA DNA Polymerase combines all the advantages of JumpStart AccuTaq LA with the added convenience of an inert red dye. This dye provides quick visual confirmation that the enzyme has been added and properly mixed. After PCR, an aliquot can be loaded directly onto an agarose gel without the need for loading buffers or tracking dyes.

### Greater Specificity and Increased Yield with JumpStart AccuTaq and JumpStart REDAccuTaq



Long and accurate hot start enzymes were used to amplify a 5 kb fragment starting with 25 ng of total human genomic DNA. All reactions were performed according to the manufacturer's specifications.

Lane M: Wide Range DNA marker (D7058)

Lane 1: JumpStart AccuTaq LA

Lane 2: JumpStart REDAccuTaq LA

Lane 3: Supplier S

Lane 4: Supplier I, enzyme P

Lane 5: Supplier I, enzyme HF

**Components:** JumpStart AccuTaq LA DNA Polymerase or JumpStart REDAccuTaq LA DNA Polymerase  
10× AccuTaq LA Buffer

**Unit definition:** One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min. at 74 °C

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
D5809	JumpStart AccuTaq LA DNA Polymerase 2.5 units per $\mu$ l	125 units 500 units 1,500 units
D1313	JumpStart REDAccuTaq LA DNA Polymerase 1 unit per $\mu$ l	50 units 250 units

# HOT START PCR

## JumpStart™ KlenTaq® LA DNA Polymerase

JumpStart KlenTaq LA DNA Polymerase is a specially blended enzyme mix containing KlenTaq-1 DNA Polymerase (a 5'-exo-minus, N-terminal deletion of Taq DNA Polymerase), a small amount of a proofreading DNA polymerase, and JumpStart Taq antibody. Blending the KlenTaq-1 with a proofreading polymerase increases the fidelity, yield and length of the amplified product. KlenTaq-1 is more efficient and more processive than either native Taq DNA Polymerase or other N-terminal deletions of Taq. KlenTaq LA has a broad magnesium optimum, so it is typically unnecessary to optimize the magnesium concentration in the reaction mixtures. It has fidelity 4× greater than that of Taq DNA Polymerase.

JumpStart Taq Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield. Unlike other hot start methods (i.e. chemical inactivation), JumpStart Taq Polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction.

JumpStart KlenTaq LA is ideal for genomic DNA amplifications of 0.5-5 kb in length and up to 20 kb on less complex templates. The antibody inactivated enzyme provides improved specificity and yield by eliminating non-specific products.

### Features and Benefits

- Produce amplicons from 0.25 to 5 kb for complex genomic DNA templates and up to 20 kb on less complex templates
- Fidelity up to 4× that of Taq DNA Polymerase
- Reduce set-up time and eliminate concerns associated with manual or wax hot start methods
- KlenTaq Polymerase works over a broad magnesium range, thereby minimizing the need for optimization

**Components:** JumpStart KlenTaq LA DNA Polymerase  
10× KlenTaq LA Buffer

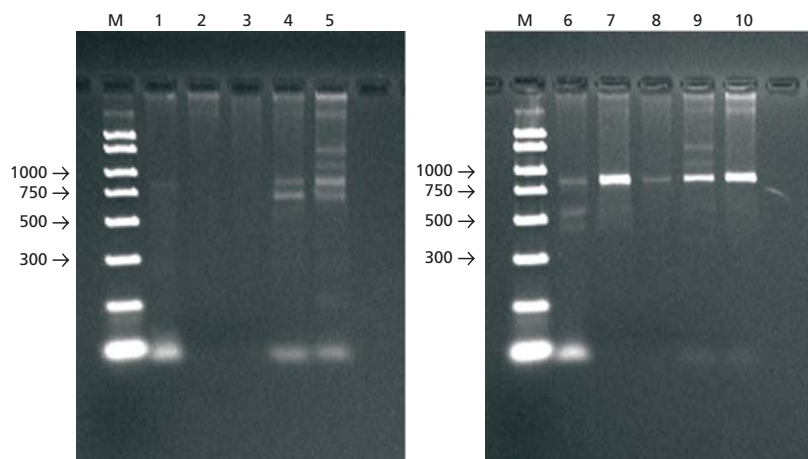
**Unit definition:** One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

**Concentration:** 1.25 units per µl

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
D0816	JumpStart KlenTaq LA DNA Polymerase	125 units 500 units 1,500 units

### Greater Yield and Specificity with JumpStart KlenTaq



Run using suppliers recommended conditions

Lane M: PCR Marker (P9577)  
Lane 1: Sigma Taq  
Lane 2: Supplier S, enzyme H  
Lane 3: Supplier Q  
Lane 4: Supplier S, enzyme Y  
Lane 5: JumpStart KlenTaq LA

Run using supplier's recommended conditions and enhancers

Lane M: PCR Marker (P9577)  
Lane 6: Taq DNA polymerase with 1 M betaine  
Lane 7: Supplier S, enzyme H with 4% DMSO  
Lane 8: Supplier Q with 1X Q solution  
Lane 9: Supplier S, enzyme Y with 4% DMSO  
Lane 10: JumpStart KlenTaq LA with 1 M betaine

JumpStart KlenTaq LA and competitors' high performance enzymes were used to amplify an 890 bp amplicon containing 80% overall G-C content from total human genomic DNA. Reactions were run according to supplier's recommendations both without (Lanes 1-5) and with (Lanes 6-10) suggested PCR enhancer.

# HOT START PCR

## JumpStart™ Taq Antibody

The primary purpose of all hot start PCR methods is to prevent Taq DNA Polymerase activity prior to thermal cycling. Even if set-up is conducted on ice, Taq DNA Polymerase remains active and may elongate unwanted products such as misprimed or other nonspecific events. Primer-dimer interactions may also occur, which will reduce overall yield and efficiency.

One method commonly used to prevent unwanted amplification products is to add JumpStart Taq Antibody to the reaction. This efficient, yet simple, procedure takes only 10 minutes and effectively inactivates the Taq DNA polymerase until the first denaturation cycle. Unlike other hot start methods (i.e. chemical inactivation), JumpStart Taq Polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction. Upon heating to 70 °C, the antibody dissociates and full activity is restored to the Taq DNA Polymerase for the remainder of the PCR. JumpStart Taq antibody works effectively on a variety of Taq based DNA Polymerases that are commercially available.

Two units of Taq DNA Polymerase are inactivated by 1 test of JumpStart Taq Antibody.

### Features and Benefits

- Minimize amplification while increasing target yield
- Reduce set-up time and eliminate concerns associated with manual or wax hot start methods

**Components:** JumpStart Taq Antibody  
Dilution Buffer for JumpStart Taq Antibody

**Storage:** -20 °C  
Shipped in wet ice

Cat. No.	Product Description	Quantity
A7721	JumpStart Taq Antibody	200 tests 500 tests