

FAQs

Ex Taq™ Polymerase

1) What is the principle of LA (Long and Accurate) PCR?

Generally, standard *Taq* DNA Polymerase has difficulty amplifying targets >5 kb. This is presumably (at least partially) due to its inability to correct dNTP misincorporations. The chain elongation rate from mismatched bases is greatly reduced, causing a decrease in the yield of longer products.

Ex Taq™ DNA Polymerase is a thermostable polymerase which possess a 3' to 5' exonuclease (proofreading) activity, allowing amplification of long targets. The 3' to 5' exonuclease activity detects and removes the misincorporated bases that cause slow elongation, making the reaction proceed smoothly, allowing generation of longer and more accurate PCR products.

2) What is the difference between *Ex Taq*™ and *LA Taq*™ DNA Polymerases?

Both the *Ex Taq*™ and *LA Taq*™ DNA Polymerase and buffer systems contain a proofreading activity. The *Ex Taq*™ system is specifically optimized for high yields and superior sensitivity, while the *LA Taq*™ system is optimized for production of longer fragments.

Ex Taq™ DNA Polymerase is generally recommended in applications where high efficiency and sensitivity are demanded in amplifications up to 20 kbp of lambda DNA and 10 kbp of human genomic DNA. *Ex Taq*™ also provides consistent results with low copy number DNA.

LA Taq™ DNA Polymerase is generally recommended for longer amplifications of up to 48 kbp on lambda DNA and 30 kbp on human genomic DNA.

3) What is the fidelity of Takara's *Ex Taq*™ DNA Polymerase?

When the mutant ratio (mutant colonies:total colonies) of *Ex Taq*™ Polymerase is compared to conventional *Taq* Polymerase using the Kunkel method, *Ex Taq*™ Polymerase shows 4-fold higher fidelity.

Note: According to the Cline method, the error rate of Takara *Taq* Polymerase is 8.7×10^{-6} .

4) Does Takara's *Ex Taq*™ DNA Polymerase produce PCR products with sticky ends (3'-A overhangs)?

All of Takara's polymerases produce products with about 80% 3' A-overhangs, and these products may be cloned into T-vectors.

5) What are some general guidelines for primers to be used with Takara's *Ex Taq*™ Polymerase?

Primer specificity is the most important consideration. For *Ex Taq*™ Polymerase, the primers should be 20-35 bases in length, with nearly 50% G+C content and balanced melting temperatures within 2-3°C of each other.

6) What is the composition of Takara's *Ex Taq*™ Polymerase buffer?

The full buffer composition of the *Ex Taq*™ buffer is proprietary, however, the magnesium concentration in the standard 10X *Ex Taq*™ buffer equals 20 mM.

Note though that because the optimal Mg^{2+} concentration in a reaction may be affected by variations in reaction mix, including concentration of dNTPs, template-primer concentrations, and chelating agents carried along with template DNA, versions of Takara *Ex Taq*™ DNA Polymerase with Mg^{2+} -free buffers are available to allow optimization.

7) What cautions should I use in handling PCR Buffers?

Repeated freeze-thawing of magnesium-containing solutions (like the 10X buffers supplied with Takara's Polymerases) may result in the formation of a fine precipitate. This precipitate can reduce the effective concentration of Mg^{2+} in the PCR reaction, thereby impairing performance. We recommend thawing the 10X Buffer at room temperature, warming gently to 37°C for 2-3 minutes, and briefly vortexing to ensure a uniform suspension.

8) Is Takara *Ex Taq*™ DNA Polymerase a LD (low DNA) enzyme?

Takara *Ex Taq*™ Polymerase is a LD enzyme - (less than or equal to 10 fg DNA), confirmed by nested PCR of *Ori* region of *E. coli* genomic DNA.

9) Can Takara's *Ex Taq*[™] Polymerase be used for combinatorial or multiplex PCR?

All of Takara's PCR polymerases can be used for both combinatorial and multiplex PCR. Combinatorial and multiplex PCR are very similar techniques, and these terms are often used interchangeably. Multiplex PCR uses one template, usually genomic DNA, and several sets of primers in the same reaction. Combinatorial PCR uses several templates and several primer sets in the same reaction.

10) What is touchdown PCR?

Touchdown PCR was originally intended to simplify the process of determining optimal primer annealing temperatures. During the initial cycles of touchdown PCR, annealing takes place at approximately 15°C above the calculated T_m . In subsequent cycles, the annealing temperature is gradually reduced by 1-2°C until it has reached approximately 5°C below the calculated T_m . Many thermal cyclers have gradient-block features, which allow touchdown PCR to be performed in a single reaction.

11) What is autosegment extension (auto-extend cycles), and when should I use it?

Autosegment extension is a technique used to increase the yield of amplification products. It is typically employed with large amplicons and is a technique used to compensate for the inactivation or depletion of reagents. At the 15th (half the total number of cycles) and subsequent cycles the extension time is extended by 15 seconds for each cycle, allowing for a significant increase in amplification efficiency in long PCR.

12) What is the composition of the Takara's *Ex Taq*[™] Polymerase Premix?

The Takara *Ex Taq*[™] Premix is at a 2X concentration, with enzyme concentrations of 0.05 U/μL each. The concentration of dNTPs is 0.4 mM for each nucleotide, with a final concentration of dNTPs in the PCR reaction of 0.2 mM each.

Some customers, particularly those performing high-throughput experiments, find the premix more convenient because it reduces the number of pipetting steps. This reduced number of pipetting steps in turn reduces the probability of error, decreases user-to-user variation, and minimizes the risk of contamination.

13) Can template quality affect amplification results?

Template quality can affect amplification results. Successful amplification of longer (>5 kb) products requires intact and highly purified template DNA. Performing an additional phenol/chloroform extraction and ethanol precipitation often resolves problems related to template quality.

14) How does *Ex Taq*[™] Hot Start DNA Polymerase differ from the standard enzymes?

Ex Taq[™] Hot Start polymerase contains a monoclonal antibody to *Taq* Polymerase, which binds to the polymerase until the temperature is elevated. The binding of this antibody prevents nonspecific amplification due to mispriming and/or formation of primer dimers during PCR reaction assembly. The antibody is then denatured in the initial PCR DNA-denaturation step, releasing the polymerase and allowing DNA synthesis to proceed. Hot Start technology results in lowered background, increased specificity, and allows room-temperature reaction assembly.

Additionally, several real-time versions of *Ex Taq*[™] Hot Start Polymerase are available (i.e. SYBR Premix *Ex Taq*[™] with ROX, *Ex Taq*[™] R-PCR (Perfect Real Time), *Ex Taq*[™] R-PCR Version 2.1, and One Step Real Time RNA PCR Kit Version 2.0) which can be used with either SYBR Green I or TaqMan[™] probes.

15) What are general reaction conditions for *Ex Taq*[™] DNA Polymerase?

The following procedure is the recommended general reaction mix for a single 50 µL reaction. To minimize the number of pipetting steps and reduce the risk of contamination, we recommend that you prepare a master mix of reagents that is sufficient for all reactions being performed.

<u>Reagent</u>	<u>Volume</u>	<u>Final Concentration</u>
Takara <i>Ex Taq</i> [™] DNA Polymerase (5 units/µl)	0.25 µL	1.25 units/50 µL
10X <i>Ex Taq</i> Buffer (Mg ²⁺ plus)	5.0 µL	1X
dNTP Mix (2.5 mM each)	4.0 µL	200 µM
Template DNA*	1-5 µL	2.5-500 ng/50 µL
Primers	1-5 µL	0.2 µM each
Sterile ddH ₂ O	up to 50 µL	

*DNA template amounts per 50 µL reaction:

Human genomic	0.1-1 µg
<i>E. coli</i> genomic or plasmid	10-100 ng
Lambda phage	0.5-2.5 ng

The following times, temperatures, and cycle numbers are starting points to use when optimizing PCR conditions for each primer pair:

94°C	30 sec	
55°C ^{&}	1 min	30 cycles
72°C	0.5-1 min/kb	

[&]The annealing temperature will need to be optimized for each primer

16) Can Takara's *Ex Taq*[™] DNA Polymerase be used to amplify GC-rich templates or those with large amounts of secondary structure?

Takara's *Ex Taq*[™] DNA Polymerase can be used for amplification of GC-rich templates or those with large amounts of secondary structure by supplementing the PCR reaction mixture with DMSO, with a final concentration of 1% DMSO.