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Product Information

JumpStart™ *Taq* ReadyMix™

Catalog Number **P2893**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

JumpStart *Taq* ReadyMix combines the performance enhancements of our JumpStart *Taq* antibody for hot start[1] PCR with the convenience of an easy-to-use reaction mixture. Since it has no added dyes, this is the ideal solution for performing high-throughput, quantitative PCR methods that rely on a fluorescent probe. This ready-to-use mixture of JumpStart *Taq* DNA polymerase, 99% pure deoxynucleotides and reaction buffer is provided in a 2x concentrate for ease-of-use. Simply add 25 μL of the 2x mix to the DNA template, primers, and water. At room temperature, the JumpStart *Taq* antibody inactivates the *Taq* DNA polymerase. When the temperature is raised above 70 °C in the first denaturation step of the cycling process, the complex dissociates and the polymerase becomes fully active. There are no special preparation steps or protocol changes required.

- The ideal ReadyMix for high throughput, quantitative PCR applications.
- For a typical PCR reaction, mix 25 μL of JumpStart *Taq* ReadyMix with 25 μL of a mixture containing template DNA, primers, and water. Reaction volumes can be scaled down, if desired.
- The hot-start mechanism using the JumpStart *Taq* antibody, which prevents non-specific product formation, allows assembled PCR reactions to be placed at room temperature for up to 2 hours without compromising the performance.
- When performing large numbers of PCR reactions, JumpStart *Taq* ReadyMix can save a significant amount of preparation time, reduce the risk of contamination from multiple pipetting steps, and provide consistent batch-to-batch and reaction-to-reaction performance.

This product has been validated in quantitative PCR, but may require supplementation with magnesium chloride solution, 25 mM, Catalog Number M8787, a suitable fluorescent probe, and, if desired, an internal reference dye, Catalog Number R4526.

Reagent

- JumpStart *Taq* ReadyMix,
  Catalog Number P2893
  20 mM Tris-HCl, pH 8.3, 100 mM KCl, 3 mM $MgCl_2$, 0.002% gelatin, 0.4 mM of each dNTP (dATP, dCTP, dGTP, TTP), stabilizers, 0.1 unit/μL *Taq* DNA Polymerase, JumpStart *Taq* antibody.
  Provided as 100 reactions and 400 reactions (50 μL reaction volume).

Reagents and equipment required, not provided

- Water, PCR Reagent, Catalog Number W1754
- Primers
- DNA template
- Dedicated pipettes
- PCR pipette tips
- 0.2 or 0.5 mL thin-walled PCR microcentrifuge tubes, Catalog Numbers P3114 or P3364, or plates for specific thermal cycler
- Thermal cycler
- Mineral Oil, Catalog Number M8662 (optional)
- DMSO, Catalog Number D8418 (optional)

Precautions and Disclaimer

JumpStart ReadyMix *Taq* 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

JumpStart Taq ReadyMix can be stored at 2–8 °C for up to 6 months so there is no waiting for the reaction components to thaw. It can also be stored at –20 °C for up to a year and a half. There was no detectable loss of performance after 10 freeze-thaw cycles.

Procedure

Optimal concentrations of template DNA, $MgCl_2$, KCl, and PCR adjuncts as well as pH are often target specific. Optimization may be required for specific template and primers. Additional components ($MgCl_2$, dNTP, KCl, or betaine) may be added to the template/primer mixture, although this is not required for most applications. The following procedure serves as a reference.

Note: DMSO (up to 5% v/v) is compatible with this system. However, other co-solvents, solutes (salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart *Taq* antibody for the *Taq* polymerase and thereby compromise its effectiveness.

1. Add the following reagents to a 0.2 mL or 0.5 mL thin-walled microcentrifuge tube or plate well.

Volume	Reagent	Final Concentration
25 μL	2x JumpStart *Taq* ReadyMix	2.5 units JumpStart *Taq* DNA polymerase
(0.5 μL)	Reference dye (optional)	(optional)
— μL	25 mM $MgCl_2$ (optional)	(optional)
1 μL	Forward primer	0.2 μM
1 μL	Reverse primer	0.2 μM
— μL	Template DNA	
— μL	Water	q.s. to 50 μL
50 μL	**Total Volume**	

Note: A template-primer master mix for each dilution of template is recommended when performing multiple PCR reactions.

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

3. Add mineral oil to the top of each tube to prevent evaporation (optional, depending on model of thermal cycler).

4. Optimal cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximal product yield and/or quality.

Typical cycling parameters for 0.2–2 kb fragments

Initial denaturation	94 °C	2 min
30-35 cycles:		
Denaturation	94 °C	30 sec
Annealing	55 °C to 68 °C	30 sec
Extension	72 °C	2 min
Final extension:	72 °C	5 min
Hold	4 °C	

References

1. Dieffenbach, C., and Dveksler, G., (Eds),. *PCR Primer: A Laboratory Manual,* 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2003.
2. Rees, W.A., *et al.*, Betaine can eliminate the base pair composition dependence of DNA melting. *Biochemistry*, **32,** 137-144 (1993).
3. Don, R.H., *et al.*, 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res*., **19,** 4008 (1991).
4. Huang, L.M., and Jeang, K.T., Long-range jumping of incompletely extended polymerase chain fragments generates unexpected products. Biotechniques, **16,** 242-246 (1994).
5. Kwok, S., and Higuchi, R., Avoiding false positives with PCR. *Nature*, **339,** 237-238 (1989).

Troubleshooting Guide

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 agarose 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.
	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.[2]
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 Tm of the primers during the initial PCR cycles. The annealing/extension temperature is then reduced to the primer Tm for the remaining PCR cycles. The change can be performed in a single step or in increments over several cycles.[3]
	Too many cycles were performed.	The nonspecific bands may be eliminated by reducing the number of cycles.
	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.

Troubleshooting Guide (continued)

Problem	Possible Cause	Solution
There is no reduction of nonspecific PCR bands when using the JumpStart *Taq* antibody.	The antibody affinity may be reduced by reaction components or conditions.	Some cosolvents, solutes (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.[4]
	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.[5]
The yield of specific product is low.	Too few cycles were performed.	Increase the cycle number in 3–5 cycle increments.
	A co-solvent is required.	Add dimethyl sulfoxide (5%) or betaine (0.8–1.3 M) to indicated 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 PCR primers.

Related Products

Reagents

- Lambda DNA *Hind* III Digest, Catalog No. D9780
- Enhanced Avian HS RT-PCR kit, Catalog No HSRT100 (100 reactions).

Equipment

- PCR Multiwell Plate, 96-well, Catalog No. Z374903
- PCR Multiwell Plate, 384-well, Catalog No. Z374911
- PCR Microtubes, 0.2 ml, attached caps, Catalog No. Z374873
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Catalog No. Z374962
- Sealing accessory for PCR vessels, Micro Mats, Catalog No. Z374938
- PCR Workstation, 120V, Catalog No. Z376213
- PCR Workstation, 240V, Catalog No. Z376221

Trademarks

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Sigma-Aldrich Co. LLC. –JumpStart™ and ReadyMix™

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