Two-step Quantitative Real-Time PCR Protocol

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I DESCRIPTION

GenScript Heat-Start™ *Taq* DNA Polymerase is a chemically modified and optimized "hot start" recombinant *Taq* DNA Polymerase. Hot starts in PCR provide increased specificity (significantly reduced background from non-specific priming), sensitivity, yield, and allow convenient set-up of PCR reactions at room temperature. GenScript Heat-Start™ *Taq* DNA Polymerase requires heat activation at 94°C for 8 min, no other modification to PCR reactions or protocols is necessary. GenScript Heat-Start™ *Taq* DNA polymerase is supplied at the concentration of 5 units per µl.

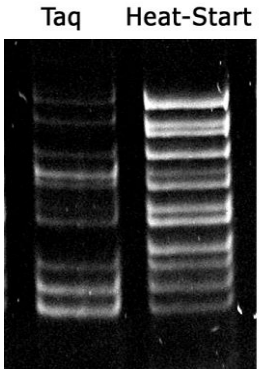
II APPLICATIONS

GenScript Heat-Start™ *Taq* polymerase can be used in most applications including the following:

- PCR, multiplex PCR, real-time PCR, RT-PCR*.
- 3' A-tailing of blunt ends.
- Primer extension.
- DNA sequencing.

III FEATURES

- Hot-start modification. GenScript Heat-Start™ *Taq* DNA polymerase is active only after the initial denaturing step. Hot starts in PCR provide increased specificity, sensitivity, yield, and allow convenient set-up of PCR reactions at room temperature.
- Terminal transferase activity. *Taq* DNA polymerase has terminal transferase activity which results in the addition of a single nucleotide (adenosine) at 3'-end of the the extension product.
- High-purity. No contamination activity has been detected in standard test reactions.



Multiplex PCR with 12 pairs of primers using Taq DNA polymerase or GenScript Heat-Start Taq DNA polymerase.

IV COMPONENT

Heat-Start™ *Taq* DNA polymerase	50 µl
10X PCR Buffer with MgCl2	1.5 ml

V UNIT DEFINITION

1 unit of Heat-Start™ *Taq* DNA polymerase incorporates 10 nmol of dNTP into acid-insoluble material in 30 min at 74 °C.

VI STORAGE

Store all the components at -20 °C

VII PCR

This protocol is suggested only as a guideline when using GenScript Heat-Start™ *Taq* DNA polymerase in PCR amplification. Optimal PCR reaction conditions vary and need to be optimized, especially in the case of multiplex PCR. PCR reaction size can be scaled up to suit user preferences.

1. Set-up PCR reaction by adding the following components to a sterile thin-wall 0.2-ml microcentrifuge tube:

10X PCR Buffer	2 µl
10 mM dNTP mix	0.4 µl
Primer mix (10 µM each)	0.4 µl
Template DNA	1 µl or more
Heat-Start™ *Taq* polymerase	0.2 µl
PCR-grade water	to 20 µl

2. Mix the contents by pipetting up and down or vortexing, then centrifuge briefly to collect the contents.
3. Perform PCR amplification as follow:

Initial denaturing step:	8 min	at 94°C
25 to 35 cycles of:		
Denaturation:	0.5 min	at 94°C
Annealing:	2 min	at 50°C -68°C depending on primer Tm
Extension:	1 min	at 72°C 1 min per kb DNA
Final extension:	3 min	at 72°C

If needed, maintain the reaction at 4°C after cycling.

4. Analyze the PCR products by agarose gel or acrylamide gel electrophoresisand visualize by ethidium bromide staining. Use appropriate DNA markers to confirm PCR DNA sizes.
5. PCR reaction can be stored at - 20°C until use.

Related Products:

Cat. No.	Product	Size	Our Price
D0056	dNTP Mixture 10 mM each	0.5 ml	$25.24
E00007	Taq DNA Polymerase	1,000 U	$60.00
D00033	Taq Supermix	100 rnxs	$89.00

Troubleshooting:

Problem	Probable Cause	Solution
No PCR DNA	One or more PCR components may be missing.	Repeat the PCR, it is recommended to use a check list every time. Check the concentrations and storage conditions of all the reagents.
	PCR conditions are not optimized. The annealing temperature may be too high; More cycles may be needed; The denaturation time may be too short; The extension time may be too short.	Optimize the PCR conditions by decreasing annealing temperature in 2-4 ^{o}C increments, or increasing the number of cycles, or increasing the denaturation time in 10 second increments, or increasing the extension time in 1minute increments. It is recommended to change one parameter each time.
	The primers may not be designed optimally.	The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Target template is highly GC-rich.	The target will be difficult to denature even with a longer denaturation step. Betaine, DMSO and formamide can help amplification of high GC-rich template.
	Genomic DNA is lost especially when a single cell is used.	Do not use pippet tips to mix. Tap the centrifuge tubes gently to mix.
Non-specific products	The primers may not be designed optimally.	Primers may form dimers, or prime at non-specific target sequences. Redesign primers.
	Annealing temperature is too low.	Optimize the PCR conditions by increasing annealing temperature in 2-4 ^{o}C increments, or decreasing the number of cycles.
High background	Too much Taq DNA polymerase may be used.	Optimize the PCR conditions by decreasing the amount of Taq DNA polymerase in 0.5 unit increments.
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

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