AccuPower® HotStart PCR PreMix

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***AccuPower*® HotStart PCR PreMix is a new, powerful, and ready-to-use PCR reagent optimized for higher PCR Specificity.**

I. Introduction

Bioneer's HotStart PCR PreMix was designed by Chemical-Mediated Hotstart method. The DNA polymerase is inhibited by the pyrophosphate, but activated upon pyrophosphate hydrolysis by the thermostable pyrophosphatase (Patent pending). This prevents the formation of mis-primed products and primer-dimers during the reaction set up process resulting in improved PCR specificity.

HotStart PCR protocols increase amplification specificity and product yield by creating conditions that minimize the possibility of nonspecific priming, primer-dimer formation and other reactions, which can occur at low temperatures once all the PCR reaction components are mixed. Unwanted side reactions occurring during the PCR process usually begin at room temperature. In general, hot-start techniques limit the availability of one essential reaction component until a higher temperature (>60°C) is reached. This can be done manually by the addition of the critical component when the reaction mixture reaches the higher temperature; however, this method is tedious and can increase the chances of contamination. Another method involves the use of a polymerase-specific antibody, which at lower temperatures, binds the enzyme and prevents polymerization. At higher temperatures, the antibody becomes unbound and releases a functional polymerase. Other techniques incorporate the critical substance in a wax bead, which melts at the higher temperature and releases the missing component.

Bioneer's HotStart DNA Polymerase is designed for hot-start PCR to provide higher PCR specificity by use of pyrophosphatase and Pyrophosphate. The combination of Hot Start DNA Polymerase and the proprietary PCR buffer minimizes nonspecific amplification products, primer–dimers, and background. It is ideal for amplification reactions involving complex genomic or cDNA templates, very low-copy targets, or multiple primer pairs.

HotStart DNA Polymerase is provided in an inactive state with no polymerase activity at ambient temperature. This prevents the formation of misprimed products and primer–dimers at low temperatures. HotStart DNA Polymerase is activated by a 5-minute, 94°C incubation step, which can easily be incorporated into existing thermal cycling programs. HotStart DNA Polymerase provides high PCR specificity and often increases the yield of the specific PCR product. Preparing the PCR mixture is quick and convenient as all the reaction components can be mixed at room temperature.

In addition, *AccuPower* ® HotStart PCR PreMix makes hot-start PCR simple and easy, eliminating the extra handling steps and contamination risks associated with conventional hot-start methods.

Notice to Purchaser

This enzyme is specifically optimized for increasing base incorporation rate by inactivating 5'->3' exonuclease activity. Therefore, this is not recommended to use for Real Time PCR using Taqman® probe, which will be released soon. Bioneer corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice. All information provided here is subject to change without notice.

II. Product Components

Description	Number of Reactions	
AccuPower® HotStart PCR PreMix [c]	K-5050[a]	K-5051[b]
	96 reactions	480 reactions

[a] Catalog K-5050 provides PCR premix kits for 96, 20 µl PCR reactions.

[b] Catalog K-5051 provides PCR premix kits for 480, 20 µl PCR reactions.

[c] The total Mg^{2+} concentration presented in the final 1 x working solution is 1.5 mM.
The total dNTP concentration presented in the final 1 x working solution is 1 mM (250 µM of each dNTP).

III. PCR Protocol using *AccuPower*® HotStart PCR PreMix

This protocol serves only as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

Notes:
- Each PCR program is sufficient to start an Pre-denaturation step of 5 min at 94°C.
- *AccuPower*® HotStart PCR PreMix provides a final concentration of 1.5 mM $MgCl_2$ in the final reaction mix, which will produce satisfactory results in most cases. However, if a higher Mg^{2+} concentration is required, prepare a stock solution containing 10 mM $MgCl_2$.
- Set up reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

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1. **Thaw primer solutions and prepare template DNA.**
2. **Distribute the appropriate volume of diluted primer mix into the each *AccuPower*® HotStart PCR PreMix**
3. **Add template DNA (≤100 ng/reaction) to the individual PCR tubes.**
   A negative control (without template DNA) should always be included. It is not necessary to keep PCR tubes on ice as nonspecific DNA synthesis cannot occur at room temperature due to the inactive state of HotStart DNA polymerase.

Table 4. Reaction composition using *AccuPower*® HotStart PCR PreMix

Component	Volume/reaction	Final concentration
***AccuPower*® HotStart PCR PreMix**	1 kit	1 unit of HotStart DNA Polymerase 1x PCR Buffer* 250 µM of each dNTP
Diluted primer mix		
Upstream primer	Variable	0.1–0.35 µM
Downstream primer	Variable	0.1–0.35 µM
Nuclease-Free water to final volume	Variable	–
Template DNA		
Template DNA, added in step 4	Variable	≤100ng/reaction
Total volume	**20 µl**	**–**

** Contains 1.5 mM $MgCl_2$*

4. **Add distilled water *to AccuPower*® HotStart PCR PreMix tubes until the total volume of mixture becomes 20 µl.**
   Dissolve the vacuum-dried blue pellet by vortexing and spin-down.
5. **When using a thermal cycler with a heated lid, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50 µl of mineral oil.**
6. **Program the thermal cycler according to the manufacturer's instructions.**
   Each PCR program is equal to Pre-denaturation step at 94°C for 5 min. A typical PCR cycling program is outlined below. For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

	Time	Temperature	Additional comments
Pre-denaturation step:	5 min	94°C	Equal to General PCR's Initial activation step
3-step cycling			
Denaturation:	~ 0.5 min	94°C	
Annealing:	0.5–1 min	50–68°C	Approximately 5°C below Tm of primers.
Extension:	~1 min	72°C	For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.
Number of cycles:	25–35		
Final extension:	5 min	72°C	

7. **Place the PCR tubes in the thermal cycler and start the cycling program.**
   Note: After amplification, samples can be stored overnight at 2–8°C or at –20°C for longer storage.
8. **Load samples on agarose gel without adding loading dye mixture and perform electrophoresis.**

IV. Ordering Information

Tube type	Reaction	Cat.No.	Description
0.2 ml Tube	20 µl	K-5050	0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes
		K-5051	0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes
	50 µl	K-5052	0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes
		K-5057	0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes
0.5 ml Tube	50 µl	K-5053	0.5 ml thin-wall tubes with attached cap / 100 tubes

A complete product list appears on our web site at ***www.bioneer.com***