Amersham™ Hot Start Master Mix

POLYMERASE CHAIN REACTION (PCR)

Amersham™ Hot Start Master Mix is a 2× premixed formulation that can effectively reduce nonspecific priming and primer-dimer formation during PCR. The system is developed based on a novel PCR method that uses a hot start activator protein to sequester primers prior to PCR, thereby making them unavailable for nonspecific priming during the reaction preparation.

Amersham™ Hot Start Master Mix is supplied as a convenient 2× master mix containing *Taq* DNA Polymerase, ultrapure deoxynucleotide triphosphates (dNTPs), hot start activator protein, and reaction buffer optimized for a wide variety of PCR applications. Additional $MgCl_2$ can be easily supplemented allowing users to customize this reagent to their specific needs. The premixed formulation saves time and reduces potential contamination errors by eliminating several pipetting steps.

Amersham™ Hot Start Master Mix offers:

- **Specificity:** reduces primer-dimer formation and nonspecific priming, and thereby increases amplification specificity and efficiency.
- **Convenience:** room temperature setup and enhanced buffer formulation reduce time-consuming optimization.
- **Reproducibility:** ready-to-use mix reduces experimental variability and can be applied to automated systems.
- **Stability:** can be subjected to repeated freeze-thaw cycles with no loss in performance.

Applications

Amersham™ Hot Start Master Mix can be readily applied to any standard PCR reaction, such as multiplex PCR, and reactions exhibiting nonspecific amplification or primer-dimer formation.

Amersham™ Hot Start Master Mix is an excellent choice for PCR amplification where primers have the potential to form dimers or exhibit nonspecific priming. Figure 1 shows PCR amplification of a 303-base pair (bp) fragment from human genomic DNA with a primer pair that has a 3-bp overlap at the 3′ ends. The PCR was performed using conventional (non-hot start) PCR master mix,

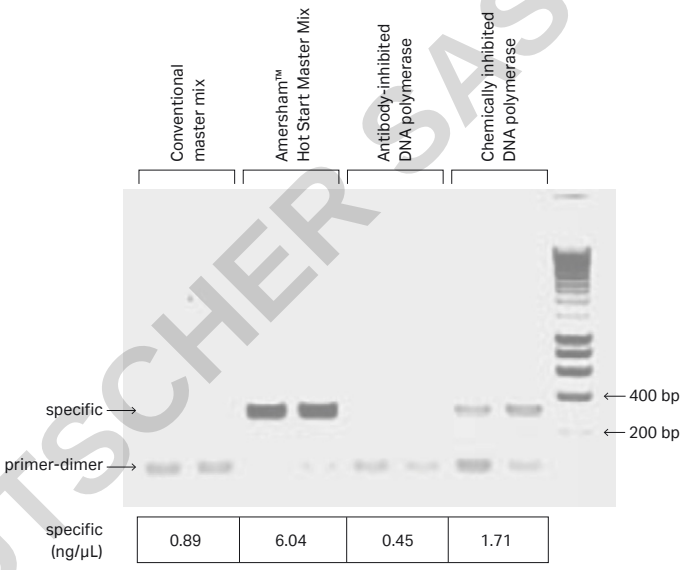


Fig 1. Human numb locus primer-dimer assay. All reactions were prepared with 1 ng of human genomic DNA as template and 0.2 µM each of forward and reverse primer. Reactions were incubated at 25°C for 60 min before cycling. All products were resolved on a 1.5% agarose-TAE gel stained with ethidium bromide. The average yield of the specific product was quantitated using an Agilent™ 2100 bioanalyzer, and is shown below the gel.

Amersham™ Hot Start Master Mix, commercially available monoclonal antibody–inactivated DNA polymerase, and chemically inactivated DNA polymerase, respectively. The results show that in the absence of hot start, no 303-bp product is formed, while most of the products are in the form of primer-dimer. However, when Amersham™ Hot Start Master Mix is used, the amplification of the 303-bp fragment is dramatically increased while the primer-dimer formation is decreased.

Components

The 2× formulation is composed of Tris-HCl, KCl, $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), *Taq* DNA Polymerase, hot start activator protein, and stabilizers. Additional $MgCl_2$ can be added to optimize the PCR conditions.

DOMINIQUE DUTSCHER SAS

Quality control

Amersham™ Hot Start Master Mix was functionally tested:

- To demonstrate prevention of primer-dimer formation during PCR amplification of numb locus fragment using oligonucleotide primers with 3-bp overlap at the 3′ terminus when compared to reactions without hot start activator protein.
- To demonstrate that polymerase activity is blocked by 90% or greater when incubated for 4 h at 25°C in the presence of DNA polymerase, dNTPs, and polymerase substrate compared to reactions without hot start activator protein.
- To ensure that Amersham™ Hot Start Master Mix is free of ribonucleases and double- and single-stranded DNA exonucleases and endonucleases. Standard assays were performed, and all of these activities were found to be negligible.

Storage

Store at -20°C.

Ordering information

Product	Pack size	Product code
Amersham™ Hot Start Master Mix	100 reactions	25150001

Related amplification products	Description/ pack size	Product code
Amersham™ Hot Start Mix in Ready-To-Go™ format	96 reactions	28900653
	100 reactions	28900646
	480 reactions	28900654
PuReTaq™ PCR Beads in Ready-To-Go™ format	0.2 mL hinged tube with cap, 96 reactions	27955901
	0.5 mL tubes, 100 reactions	27955801
	Multiwell plate, 96 reactions	27955701
	Multiwell plate, 5× 96 reactions	27955702
Amersham™ RT-PCR Beads in Ready-To-Go™ format	0.2 mL hinged tube with cap, 96 reactions	27925901
	0.2 mL tube, 96 reactions	27926701
	0.5 mL tube, 100 reactions	27926601

Related cleanup products	Pack size	Product code
ExoProStar™ PCR Clean-Up Technology	20 reactions	US78220
	100 reactions	US78210
	500 reactions	US78211
	2000 reactions	US78212
	5000 reactions	US78225
GFX™ PCR DNA and Gel Band Purification Kit	96 purifications	28903445
MicroSpin™ G-25 Columns	50 purifications	27532501
MicroSpin™ G-50 Columns	50 purifications	27533001
	250 purifications	27533002
MicroSpin™ S-200 HR Columns	50 purifications	27512001
MicroSpin™ S-300 HR Columns	50 purifications	27513001
MicroSpin™ S-400 HR Columns	50 purifications	27514001

DOMINIQUE DUTSCHER SAS

cytiva.com

For local office contact information, visit cytiva.com/contact

Cytiva and the Drop logo are trademarks of Life Sciences IP Holdings Corp. or an affiliate doing business as Cytiva. Amersham, ExoProStar, GFX, MicroSpin, Ready-To-Go, and PureTaq are trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

Agilent is a trademark of Agilent Technologies, Inc. Any other third-party trademarks are the property of their respective owners.

© 2022 Cytiva

CY28492-24May22-DF

cytiva