Polymerase chain reaction (PCR)

illustra™ Hot Start Master Mix

illustra 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.

illustra 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.

illustra 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

illustra 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.

illustra 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,

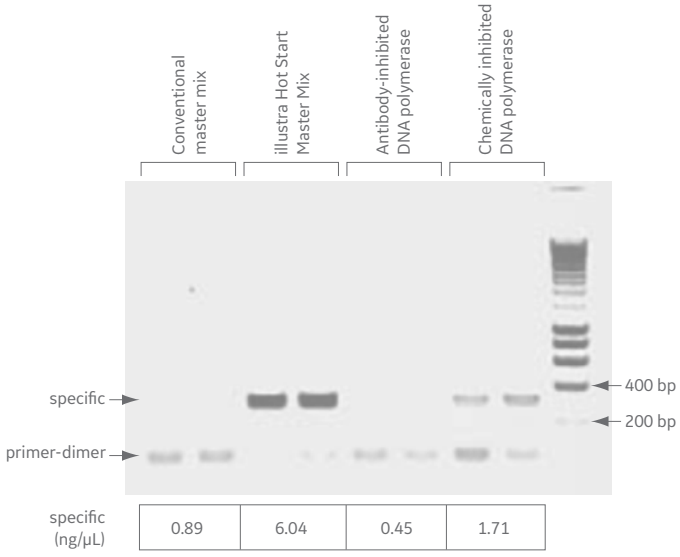

Fig 1. Human numb locus primer-dimer assay. All reactions were prepared with 1 ng of human genomic DNA as template and 0.2 µM each of forward and reverse primer. Reactions were incubated at 25°C for 60 min before cycling. All products were resolved on a 1.5% agarose-TAE gel stained with ethidium bromide.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

illustra 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 illustra 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.

Quality control

illustra 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 illustra 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	Description	Product code
illustra Hot Start Mix	100 reactions	25150001
Related amplification products	**Pack size**	**Product code**
illustra Hot Start Mix RTG™	96 reactions	28900653
illustra Hot Start Mix RTG	100 reactions	28900646
illustra Hot Start Mix RTG	480 reactions	28900654
illustra PuReTaq Ready-To-Go™ PCR Beads	0.2 mL hinged tube with cap, 96 reactions	27955901
illustra PuReTaq Ready-To-Go PCR Beads	0.5 mL tubes, 100 reactions	27955801
illustra PuReTaq Ready-To-Go PCR Beads	Multiwell plate, 96 reactions	27955701
illustra PuReTaq Ready-To-Go PCR Beads	Multiwell plate, 5× 96 reactions	27955702
illustra Ready-To-Go RT-PCR Beads	0.2 mL hinged tube with cap, 96 reactions	27925901
illustra Ready-To-Go RT-PCR Beads	0.2 mL tube, 96 reactions	27926701
illustra Ready-To-Go RT-PCR Beads	0.5 mL tube, 100 reactions	27926601
Related cleanup products	**Pack size**	**Product code**
illustra ExoProStar™	20 reactions	US78220
illustra ExoProStar	100 reactions	US78210
illustra ExoProStar	500 reactions	US78211
illustra ExoProStar	2000 reactions	US78212
illustra ExoProStar	5000 reactions	US78225
illustra GFX™ PCR DNA and Gel Band Purification Kit	96 purifications	28903445
illustra MicroSpin™ G-25 Columns	50 purifications	27532501
illustra MicroSpin G-50 Columns	50 purifications	27533001
illustra MicroSpin G-50 Columns	250 purifications	27533002
illustra MicroSpin S-200 HR Columns	50 purifications	27512001
illustra MicroSpin S-300 HR Columns	50 purifications	27513001
illustra MicroSpin S-400 HR Columns	50 purifications	27514001

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