



## 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.

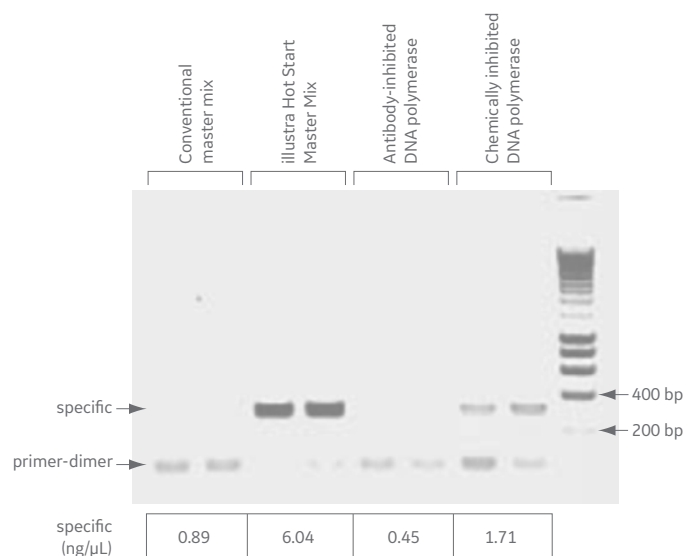
### illustra Hot Start Master Mix offers:

- **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

<b>Product</b>	<b>Description</b>	<b>Product code</b>
illustra Hot Start Mix	100 reactions	25150001
<b>Related amplification products</b>		
<b>Pack size</b>	<b>Product code</b>	
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
<b>Related cleanup products</b>		
<b>Pack size</b>	<b>Product code</b>	
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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