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LA Hot Start Master Mix

(Catalogue number L608, L609, L610, L610xl) rev. 01/2022

Description

LA Hot Start Master Mix is optimized mix for universal use in PCR. It contains a blend of two DNA polymerases, deoxyribonucleotide triphosphates (dNTPs), Dye, anti-Taq monoclonal antibody, buffer, stabilizers, additives and other components. **This product is the most advanced variant of Master mix for PCR:**

Rapid samples preparation, 500 µl aliquots

- 2x concentrated Master Mix is in 500 µl aliquots. This decreases possibility of contamination and allows rapid preparation of the samples by adding just specific oligonucleotide primers, template DNA and H_2O (included).

LA (Long and Accurate)

- The Mix contains unique blend of two thermostable DNA polymerases. One of them is Taq DNA polymerase, which is highly processive but lacks a 3'->5' exonuclease proofreading activity, which accounts for relatively high error rate during DNA amplification. Second polymerase is less processive but possesses 3'->5' exonuclease activity necessary for proofreading. The polymerases mix decreases error rate during synthesis of complementary DNA strands and preserves high speed of DNA synthesis. The repair capability allows amplification of complex genomic DNA fragments (up to 15 kbps in size) and less complex viral DNA (up to 40 kbps in size) and significantly decreases error rate when compared to Taq DNA polymerase

Hot Start

- The Mix contains monoclonal antibody, which binds DNA polymerases and thereby blocks their enzymatic activity and amplification of nonspecific DNA fragments. After the first denaturation cycle, the antibody is irreversibly inactivated and Taq DNA polymerase regains enzymatic activity.

Direct loading into the gel

- The Mix contains additives and a dye, which allow direct loading of the samples into the gel without necessity of adding loading buffer.
- Dye present in the Mix migrates in the agarose gel in front of the primers and therefore does not interfere with quantification of the PCR products. The dye and other additives have no effect on DNA amplification during PCR.

High efficiency and specificity

- The Mixt allows highly sensitive and specific amplification of corresponding fragments of DNA or cDNA; it possesses $MgCl_2$ at a concentration suitable for most PCRs.

Technical data

Components and packaging

- 1 tube with 0.5 ml LA Hot Start Master Mix (for 40 reactions, 25 µl each).
- 1 tube with 1.5 ml PCR H_2O.

Composition

- 2x concentrated LA Hot Start Master Mix contains: 150 mM Tris-HCl, pH 8.8 (at 25°C), 40 mM $(NH_4)_2SO_4$, 0.02% Tween 20, 5 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP, 100 U/ml Taq DNA polymerase blend, monoclonal antibody anti-Taq (38 nM), dye, stabilizers and additives.

Storage

- At temperature -20°C ± 5°C; shortly (up to one week) at temperature 4 - 30°C. This decreases demands for transportation (Nature friendly). Material can be repeatedly defrosted.

Purity and quality control

- Purity of Taq DNA polymerases is verified electrophoretically (SDS PAGE). Material is free of nucleases.
- Each batch of LA Hot Start Master Mix is tested for amplification of a single copy gene in genomic DNA.

Cat. No	Product name and specification	Quantity
L608	LA Hot Start Master Mix (1x)	40 reactions
L609	LA Hot Start Master Mix (5x)	200 reactions
L610	LA Hot Start Master Mix (25x)	1000 reactions
L610xl	LA Hot Start Master Mix (100x)	4x 1000 reactions

Protocol

Suggested basic protocol for PCR amplification using LA Hot Start Master Mix

1. In a thin-walled PCR tube the following components are mixed:

Volume*	Reagent	Final concentration
12.5 µl	LA Hot Start Master Mix	1x LA Hot Start Master Mix (75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2.5 mM $MgCl_2$, 200 µM dATP, 200 µM dCTP, 200 µM dGTP , 200 µM dTTP, 50 U/ml blend of DNA polymerases, 19 nM anti-Taq monoclonal antibody)
1 µl	5' primer	0.1 - 1 µM (~ 20 bases)
1 µl	3' primer	0.1 - 1 µM (~ 20 bases)
1 µl	Template DNA	
9.5 µl	PCR H_2O	to a final volume 25 µl

*Different volumes can be used, but Master Mix should be finally diluted twice.

2. Mix gently and briefly centrifuge.

3. Add ~20 µl of PCR oil (Cat. No. P043) to prevent evaporation (this is not required if thermal cycler with a heated lid is used).

4. Perform PCR under conditions optimized for the primers used. Common cycling parameters are:

	Temperature	**Time**	**Number of cycles**
Initial denaturation	94°C	1 min	1
Denaturation Primers annealing Extension	94°C 55-68°C [1] 72°C	15 s 15 s 1 min per 1 kb	25-35
Final extension	72°C	7 min	1
Cooling	22°C		

[1] Should be determined experimentally; usually 5°C below melting temperature of the primers.

5. Amplified DNA can be directly loaded into agarose gel without adding loading buffer.