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LA DNA Polymerases Mix

(Catalogue number L072, L073, L074) rev. 01/2022

Description

LA DNA polymerases Mix is 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 the polymerases to resume elongation of the growing DNA strand and amplification of complex genomic DNA fragments up to 20 kbps. Using less complex templates, such as bacterial genomic DNA or viral DNA, amplifications of up to 20 kbps or 40 kbps, respectively, have been achieved. The fidelity of LA DNA Polymerases Mix is approximately 5 times greater than Taq DNA polymerase. The polymerases mix is intended for "**L**ong and **A**ccurate" (**LA**) PCR. It is especially suitable for amplification of DNA fragments designated for cloning purposes. Importantly, the polymerases blend creates DNA amplicons with mostly blunt ends as a result of the proofreading activity of the polymerases.

Technical data

Components and packaging

- LA DNA polymerases Mix is supplied at a concentration 5 U/µl. Basic packaging is 1 tube with 500 U/100 µl (Cat. No. L072), 5 tubes with 500 U/100 µl (L073) or 10 tubes with 500 U/100 µl (L074).
- Each test tube of LA DNA polymerases Mix is accompanied with a test tube with 10x concentrated LA PCR reaction buffer containing $MgCl_2$ (1.5 ml). If different concentration of $MgCl_2$ is required, a test tube with 10x concentrated LA PCR reaction buffer without $MgCl_2$ (1.5 ml) and a separate test tube with 25 mM $MgCl_2$ (0.5 ml) should be ordered (Cat. No. L076).
- Each package of LA DNA polymerases mix also contains 1 test tube with DMSO (1 ml).

Storage

- At temperature -20°C ± 5°C. Material can be repeatedly defrosted.

Composition

- Storage buffer for LA DNA polymerases Mix: 20 mM Tris-HCl, pH 8.0 (25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Nonidet P-40, 0.5% Tween 20, 50% glycerol.
- 10x LA PCR reaction buffer: 500 mM Tris-HCl (pH 9.3 at 25°C), 150 mM $(NH_4)_2SO_4$, 1% Tween 20, 22.5 mM $MgCl_2$.

Activity

- One unit is defined as the amount of enzyme, which catalyzes incorporation of 10 nmol dNTPs within 30 min at 72°C into trichloracetic acid precipitable material. Reaction conditions are as follow: 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 200 µM dATP, dCTP, dGTP and [α-^{32}P]dTTP, 50 µg/ml denatured cDNA, 0.5 µM primer and 0.2 – 0.5 U of enzyme in the volume of 50 µl.

Purity and quality control

- Purity of the polymerases is tested by SDS-PAGE. Material is free of nucleases.
- Each batch of LA DNA polymerases Mix is tested for its ability to amplify DNA fragment from mammalian genomic DNA by PCR. The results are verified by electrophoresis in agarose gel in the presence of ethidium bromide; only DNA band of the expected size is present.

Cat. No.	Product name and specification	Amount
L072	LA DNA polymerases Mix	500U
L073	LA DNA polymerases Mix	5x 500U
L074	LA DNA polymerases Mix	10x 500U

L076 ml	10x conc. Taq buffer without $MgCl_2$+$MgCl_2$	1.5 ml + 0.5

LA DNA Polymerases Mix

(Catalogue number L072, L073, L074)

Protocol

Basic protocol

The protocol described below can be used for routine LA PCR. However, in some cases reaction conditions must be optimized, mainly annealing temperature and concentration of $MgCl_2$.

1. In thin-wall test tube the following components are mixed[1]:

	PCR in 50 µl	Final concentration
10x LA PCR reaction buffer with $MgCl_2$[2]	5 µl	1x react. buffer with 1.5 mM $MgCl_2$
PCR dNTP mix (10 mM each) (Cat. No. P041)	2.5 µl	0.5 mM dNTP each
DMSO[3]	1 µl	2%
5´ primer (50 µM)	0.5 µl	0.5 µM
3´ primer (50 µM)	0.5 µl	0.5 µM
LA DNA polymerases Mix (5 U/µl)	0.5 ul	2.5 U (0.05 U/µl)
Template DNA (1 ng/µl - 1 µg/µl)[4]	1 ul	0.02 ng/µl – 0.02 µg/µl
PCR H_2O (Cat. No. P042)	39 ul	

[1] When more DNA samples are tested with the same primers, it is convenient to prepare so called Master Mix in which the volumes of individual components are multiplied by the number of DNA samples tested. Then, 49 µl aliquots of the Master Mix are distributed into the PCR tubes, followed by addition of tested DNA (1 µl).

[2] If unsatisfactory results are obtained, PCRs with different concentrations of $MgCl_2$ should be performed (see below).

[3] DMSO decreases formation of secondary structures and thus enhances efficiency of PCR.

[4] When amplified long DNA fragments, template DNA should be prepared carefully and should not be repeatedly defrosted.

2. Samples are gently homogenized (without vortexing) and spun down.
3. If cycler without heating lid is used, PCR oil 25 µl (Cat. No. P043) is added.
4. Thermal cycler is programmed according to the manufacturer's instructions. A typical cycling program is:

	Temperature	Time	Number of cycles
Initial denaturation	94°C	3 min	1
Denaturation	94°C	30 s	25-35
Annealing of primers	55-68°C[1]	30 s	
Extension	72°C	1 min per 1 kb	
Final extension	72°C	10 min	1
Cooling	4°C		

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

5. After PCR, samples are mixed with loading buffer (Cat. No. P048, P062, P066 or P065) and analyzed by electrophoresis in agarose gel in the presence of ethidium bromide (Cat. No. P046). Alternatively, the samples can be stored at -20°C.

Optimization of $MgCl_2$ concentration

Enhanced concentration of $MgCl_2$ (2.25 mM) in LA PCR reaction buffer is used because of enhanced concentration of dNTPs, which exhibit chelating properties; this concentration of $MgCl_2$ is suitable for most PCRs. However, if amplification of nonspecific DNA fragments is observed, optimal Mg^{2+} concentration for given PCR should be determined. To this end 10x reaction buffer without $MgCl_2$ and 25 mM $MgCl_2$ should be used (Cat. No. L076).

1. Preparation of Master Mix without $MgCl_2$ by mixing the following components:

10x LA reaction buffer without $MgCl_2$	40 µl
PCR dNTP mix (10 mM each)	20 µl
DMSO	8
5´ primer (50 µM)	4 µl
3´ primer (50 µM)	4 µl
LA DNA polymerases Mix (5U/µl)	4 µl
Template DNA (1 ng/µl - 1 µg/µl)	8 µl
PCR H_2O	232 µl
Total volume	**320 µl**

2. Master Mix is thoroughly but gently mixed, centrifuged briefly and 40 µl aliquots are distributed into 7 PCR tubes.
3. 25 mM $MgCl_2$ and PCR H_2O is added into individual test tubes with PCR Master mixes as follows (total volume 50 µl):

Tube No.	**25 mM $MgCl_2$**	**PCR H_2O**	**Final $MgCl_2$ concentration**
1	2 µl	8 µl	1.0 mM
2	3 µl	7 µl	1.5 mM
3	4 µl	5 µl	2.0 mM
4	5 µl	5 µl	2.5 mM
5	6 µl	4 µl	3.0 mM
6	8 µl	2 µl	4.0 mM
7	10 µl	0 µl	5.0 mM

4. PCR is performed as above and the samples are analyzed by electrophoresis in agarose gel in the presence of ethidium bromide. Optimal concentration of $MgCl_2$ for given PCR is thus determined.