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Combi PPP Master Mix

(Cat. No. C208, C209, C210, C210xl) rev. 01/2022

Description

Combi PPP Master Mix is dedicated for simplified routinely performed hot start PCR. It contains Taq DNA polymerase, deoxyribonucleotides, reaction buffer components, additives and monoclonal antibody anti-Taq. Samples for PCR are prepared by simple mixing Combi PPP Master Mix with target specific oligonucleotide primers, template DNA and water. Additives and the dye present in Combi PPP Master Mix allow direct loading of the PCR amplified samples into the gel without adding loading buffer. When compared to PPP Master Mix (Cat. No, P124 - P126), it contains a **monoclonal antibody** which binds to and inactivates enzymatic activity of Taq DNA polymerase. The master mix is not recommended for any applications in which fluorescence excitation is used, such as qPCR.

Hot start

- Combi PPP Master Mix contains anti-Taq DNA polymerase monoclonal antibody which inactivates enzymatic activity of the enzyme. After the first denaturation cycle, the antibody is irreversibly inactivated and Taq DNA polymerase regains enzymatic activity.

Rapid samples preparation

- All components of the Combi PPP Master Mix are 2x concentrated, which facilitates rapid preparation of the PCR samples. The samples are prepared by mixing an aliquot of Combi PPP Master Mix with oligonucleotide primers, template DNA and H_2O (included).
- Combi PPP Master Mix is especially useful for routine analyses of large numbers of DNA samples. To 0.5 ml of the Master Mix in original tube, primers (e.g. 40 µl forward and 40 µl reverse) and PCR H_2O (380 µl) are added and mixed; the "armed" Mix can be stored at -20 ± 5°C. Immediately before use, the Mix is thawed and each 24 µl aliquot is mixed with 1 µl of the tested DNA template and PCR is performed.

Direct loading into the gel

- Combi PPP Master Mix contains additives and a dye which allow direct loading of the samples into the gel, without necessity to add 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 kit allows highly sensitive and specific amplification of corresponding DNA fragments from genomic DNA or from cDNA obtained by reverse transcription; it possesses $MgCl_2$ at a concentration suitable for most PCRs.
- Monoclonal antibody bound to the enzyme significantly reduces production of nonspecific PCR products.

Technical data

Components and packaging

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

Composition

- 2x concentrated Combi PPP 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, monoclonal antibody anti-Taq (38 nM), dye, stabilizers and additives.

Storage

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

Purity and quality control

- Purity of Taq DNA polymerase is verified by SDS PAGE, only one band of 94 kDa is observed in Coomassie blue stained gel. Material is free of nucleases.
- Each batch of Combi PPP Master Mix is tested for amplification of a single copy gene in genomic DNA.

Protocol

Suggested basic protocol for PCR amplification using Combi PPP Master Mix

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

Volume*	Reagent	Final concentration
12.5 µl	Combi PPP Master Mix	1x Combi PPP Master Mix (75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 200 µM dATP, 200 µM dCTP 200 µM dGTP, 200 µM dTTP, 2.5 U Taq DNA polymerase, monoclonal antibody anti-Taq, stabilizers and additives)
1 µl	5' primer	0.1 - 1 µM (~ 20 bases in length)
1 µl	3' primer	0.1 - 1 µM (~ 20 bases in length
1 µl	Template DNA	
9.5 µl	PCR H_2O	to a final volume 25 µl

*Different volumes can be used, but Combi PPP 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 Annealing of primers 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.

Cat. No.	Product name and specification	Quantity
C208	Combi PPP Master (1x)	40 reactions
C209	Combi PPP Master (5x)	200 reactions
C210	Combi PPP Master (25x)	1000 reactions
C210xl	Combi PPP Master (100x)	4x 1000 reactions