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Plain Combi PPP Master Mix

(Cat. No. C211, C212, C213, C213xl) rev. 04/2025

Description

Plain Combi PPP Master Mix is a modification of Combi PPP Master mix (Cat. No. C208 - C210) **not containing loading dye**. This Mix is first choice for those applications where loading dye would interfere with measurements of DNA products e.g. by capillary chromatography or real-time PCR. The mix contains Taq DNA polymerase, deoxyribonucleotides, reaction buffer components, additives and anti-Taq DNA polymerase.

Hot start

- Plain 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. This reduces amplification of nonspecific products .

Rapid preparation of PCR samples

- All components of the Plain Combi PPP Master Mix are 2x concentrated, which facilitates rapid preparation of the PCR samples. The samples are prepared by mixing an aliquot of Plain Combi PPP Master Mix with oligonucleotide primers, template DNA and H_2O (included). For qPCR analysis it is necessary to add fluorescent DNA-binding dye (such as SYBR Green I) or TaqMan probes.
- Plain 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

- Plain Combi PPP Master Mix contains additives which allow direct loading of the samples into the gel, without necessity to add loading buffer.

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

Composition

- 2x concentrated Plain 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), 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 Plain Combi PPP Master Mix is tested for amplification of a single copy gene in genomic DNA.

Cat. No.	Product name and specification	Quantity
C211	Plain Combi PPP Master (1x)	40 reactions
C212	Plain Combi PPP Master (5x)	200 reactions
C213	Plain Combi PPP Master (25x)	1000 reactions
C213xl	Plain Combi PPP Master (100x)	4x 1000 reactions

Protocol

Suggested basic protocol for PCR amplification using Plain Combi PPP Master Mix

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

Volume*	Reagent	Final concentration
12.5 µl	Plain Combi PPP Master Mix	1x Plain 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 Plain Combi PPP Master Mix should be finally diluted twice. Various fluorescent dyes (e. g. SYBR Green I or TaqMan probes) can be also added but amount of H_2O is reduced accordingly.

2. Mix gently and briefly centrifuge.

3. Add ~20 µl of PCR oil (Cat. No. P 043) 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 loaded into agarose gel after adding a loading buffer (Cat. No P048, P062, P064 or P066).