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PPP Master Mix

(Cat. No. P124, P125, P126, P126xl) rev. 01/2022

Description

PPP Master Mix is dedicated for simplified routinely performed PCR. It contains Taq DNA polymerase, deoxyribonucleotides, reaction buffer components and additives.
Samples for PCR are prepared by simple mixing PPP Master Mix with target specific oligonucleotide primers, template DNA and water. Additives and a dye present in the PPP Master Mix allow direct loading of the PCR amplified samples into the gel without adding loading buffer. The master mix is not recommended for any applications in which fluorescence excitation is used, such as qPCR.

Rapid samples preparation

- All components of the PPP Master Mix are 2x concentrated, which facilitates rapid preparation of the PCR samples. The samples are prepared by mixing an aliquot of PPP Master Mix with oligonucleotide primers, template DNA and water (included).
- PPP Master Mix is extremely useful for routine analysis of large sample numbers. To 0.5 ml of the Master Mix in original tube, primers and PCR H_2O is added and the Mix can be stored at -20°C. Immediately before PCR, the Mix is thawed, aliquoted and DNA templates are added.

Direct loading into the gel

- 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 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.

Optimized reaction buffer

- Reaction buffer in the Mix enhances specificity and efficiency of PCR; it contains $MgCl_2$ at a concentration optimized for majority of PCR.
- Stabilizers present in the Mix allow its storage for short periods (weeks) at 2 - 8°C or at room temperature.

Technical data

Components and packaging

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

Composition

- 2x concentrated PPP Master Mix contains 150 mM Tris-HCl, pH 8.8 (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, dye, stabilizers and additives.

Storage

- For short terms (days) at 4°C ± 3°C.
- For long terms at -20 ± 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 PPP Master Mix without $MgCl_2$ is tested for amplification of a single copy gene in genomic DNA.

Cat. No.	Product name and specification	Quantity
P124	PPP Master (1x)	40 reactions
P125	PPP Master (5x)	200 reactions

P126	PPP Master (25x)	1000 reactions
P126xl	PPP Master (100x)	4x 1000 reactions

Protocol

Suggested protocol for PCR amplification using PPP Master Mix

1. Mix the following reagents in a thin-walled PCR tube

Volume*	Reagent	Final concentration
12.5 µl	PPP Master Mix	1x 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 Purple DNA polymerase, stabilizers and additives)
1 µl	Forward primer	0.1 - 1 µM (~ 20 bases in length)
1 µl	Reverse 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 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.

Protocol for "hot start" PCR (to decrease amplification of unwanted targets)

1. Mix the following reagents in a thin-walled PCR tube:

1 µl Forward primer
1 µl Reverse primer
1 µl Template DNA
9.5 µl PCR H_2O.

2. Mix gently and briefly centrifuge.

3. Warm to 94°C, 1 min (denaturation).

4. Add 12.5 µl PPP Master Mix, mix gently.

5. 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).

6. Perform PCR and analyze samples as described above.