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PPP Master Mix without $MgCl_2$

(Cat. No. P134, P135, P136, P136xl) rev. 01/2022

Description

PPP Master Mix without $MgCl_2$ is similar to PPP Master Mix (cat. No. P124 - P126) but does not contain $MgCl_2$, which is provided separately. This allows optimization of Mg^{2+} concentration, which is of key importance for proper PCR. The Mix contains Taq DNA polymerase, deoxyribonucleotides, reaction buffer components and additives.

Rapid samples preparation

- All components of the PPP Master Mix without $MgCl_2$ 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. $MgCl_2$ (included) and water (included).

Direct loading into the gel

- PPP Master Mix without $MgCl_2$ 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 a buffer, which after addition of proper amount of $MgCl_2$ is optimal for majority of PCRs.
- Stabilizers present in the Mix allow its storage for short periods at 4°C ± 3°C (days).

Technical data

Components and packaging

- 1 tube with 0.5 ml PPP Master Mix without $MgCl_2$ (for 40 reactions, 25 µl each).
- 1 tube with 0.5 ml 25 mM $MgCl_2$.
- 1 tube with 1.5 ml PCR H_2O.

Composition

- 2x concentrated PPP Master Mix without $MgCl_2$ contains: 150 mM Tris-HCl, pH 8.8 (at 25°C), 40 mM $(NH_4)_2SO_4$, 0.02% Tween 20, 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.

PPP master mix without $MgCl_2$ is extraordinarily suitable for routine testing of large number of samples.
It is possible to prepare complete master mix by adding the required primers (e.g. 40 µl of forward primers and 40 µl of reverse primer) directly to the original tube with PPP master mix without $MgCl_2$ (0.5 ml), optimal concentration of $MgCl_2$ and PCR H_2O up to 960 µl. Preparation of PCRs can be then restricted to mixing the complete PPP master mix (24 µl) and analyzed DNA sample (1 µl).

Cat. No.	Product name and specification	Quantity
P134	PPP Master Mix without $MgCl_2$ (1x)	40 reactions
P135	PPP Master Mix without $MgCl_2$ (5x)	200 reactions
P136	PPP Master Mix without $MgCl_2$ (25x)	1000 reactions
P136xl	PPP Master Mix without $MgCl_2$ (100x)	4x 1000 reactions

Protocol

Suggested protocol for PCR amplification using PPP Master Mix without $MgCl_2$

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

Volume*	Reagent	Final concentration
12.5 µl	PPP Master Mix without $MgCl_2$	1x PPP Master Mix without $MgCl_2$ (75 mM Tris-HCl, pH 8.8 (at 25°C), 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, stabilizers and additives)
2.5 µl	25 mM $MgCl_2$	2.5 mM $MgCl_2$
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	
7 µl	PCR H_2O	to a final volume 25 µl

*Different volumes can be used, but PPP Master Mix without $MgCl_2$ 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.

Determination of optimal concentration of $MgCl_2$

If amplification of nonspecific DNA fragments is observed under standard PCR (with 2.5 mM $MgCl_2$), we recommend to verify first whether optimal Mg^{2+} concentration is used.

1. Prepare final PPP Master Mix without $MgCl_2$ by mixing the following reagents (for 9 tubes):

112.5 µl PPP Master Mix without $MgCl_2$
9 µl 5' primer
9 µl 5' primer
9 µl template DNA

Mix briefly, centrifuge and aliquot 15.5 µl into PCR tubes marked M1 – M8.

2. Add into individual tubes with 15.5 µl final PPP Master Mix without $MgCl_2$:

Tube No.	25 mM $MgCl_2$	PCR H_2O	Final concentration of $MgCl_2$ in PCR mixture
1	5.0 µl	4.5 µl	5.0 mM
2	4.0 µl	5.5 µl	4.0 mM
3	3.0 µl	6.5 µl	3.0 mM
4	2.5 µl	7.0 µl	2.5 mM
5	2.0 µl	7.5 µl	2.0 mM
6	1.5 µl	8.0 µl	1.5 mM
7	1.0 µl	8.5 µl	1.0 mM
8	0.5 µl	9.0 µl	0.5 mM

3. Perform PCR and usual analysis of samples for determination of optimal $MgCl_2$ concentration.