

## PPP Master Mix without MgCl<sub>2</sub>

(Cat. No. P134, P135, P136, P136xl)

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### Description

PPP Master Mix without MgCl<sub>2</sub> is similar to PPP Master Mix (cat. No. P124 - P126) but does not contain MgCl<sub>2</sub>, which is provided separately. This allows optimization of Mg<sup>2+</sup> 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<sub>2</sub> 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<sub>2</sub> (included) and water (included).

### **Direct loading into the gel**

- PPP Master Mix without MgCl<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> (for 40 reactions, 25 µl each).
- 1 tube with 0.5 ml 25 mM MgCl<sub>2</sub>.
- 1 tube with 1.5 ml PCR H<sub>2</sub>O.

### **Composition**

- 2x concentrated PPP Master Mix without MgCl<sub>2</sub> contains: 150 mM Tris-HCl, pH 8.8 (at 25°C), 40 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 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<sub>2</sub> is tested for amplification of a single copy gene in genomic DNA.

### **PPP master mix without MgCl<sub>2</sub> 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<sub>2</sub> (0.5 ml), optimal concentration of MgCl<sub>2</sub> and PCR H<sub>2</sub>O 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 <sub>2</sub> (1x)	40 reactions
P135	PPP Master Mix without MgCl <sub>2</sub> (5x)	200 reactions
P136	PPP Master Mix without MgCl <sub>2</sub> (25x)	1000 reactions
P136xl	PPP Master Mix without MgCl <sub>2</sub> (100x)	4x 1000 reactions



## Protocol

### Suggested protocol for PCR amplification using PPP Master Mix without MgCl<sub>2</sub>

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

Volume*	Reagent	Final concentration
12.5 µl	PPP Master Mix without MgCl <sub>2</sub>	1x PPP Master Mix without MgCl <sub>2</sub> (75 mM Tris-HCl, pH 8.8 (at 25°C), 20 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 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 <sub>2</sub>	2.5 mM MgCl <sub>2</sub>
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 <sub>2</sub> O	to a final volume 25 µl

\*Different volumes can be used, but PPP Master Mix without MgCl<sub>2</sub> 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	94°C	15 s	25-35
Annealing of primers	55-68°C <sup>1</sup>	15 s	
Extension	72°C	1 min per 1 kb	
Final extension	72°C	7 min	1
Cooling	22°C		

<sup>1</sup>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<sub>2</sub>

If amplification of nonspecific DNA fragments is observed under standard PCR (with 2.5 mM MgCl<sub>2</sub>), we recommend to verify first whether optimal Mg<sup>2+</sup> concentration is used.

1. Prepare final PPP Master Mix without MgCl<sub>2</sub> by mixing the following reagents (for 9 tubes):

112.5 µl PPP Master Mix without MgCl<sub>2</sub>

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<sub>2</sub>:

Tube No.	25 mM MgCl <sub>2</sub>	PCR H <sub>2</sub> O	Final concentration of MgCl <sub>2</sub> 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<sub>2</sub> concentration.