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Plain PP Master Mix

(Cat. No. P201, P202, P203, P203xl) rev. 04/2025

Description

Plain PP Master Mix is similar to PPP Master Mixes (Cat. No. P124 - P126) but does not contain a dye. Therefore, the Plain PP master Mix is extraordinarily suitable for routine PCR analysis by automatic detectors (capillary chromatography, real-time PCR and others). The mix contains Taq DNA polymerase, deoxyribonucleotides, reaction buffer components and additives. Samples for PCR are prepared by simple mixing Plain PP Master Mix with target specific oligonucleotide primers, template DNA and water. For qPCR analysis it is necessary to add fluorescent DNA-binding dye (such as SYBR Green I) or TaqMan probes.
For gel electrophoresis analysis it is recommended to add a loading buffer (Cat. No P048, P062, P064 or P066).

Rapid samples preparation

- All components of the Plain PP Master Mix are 2x concentrated, which facilitates rapid preparation of the PCR samples. The samples are prepared by mixing an aliquot of Plain PP Master Mix with oligonucleotide primers, template DNA, H_2O (included) and if required fluorescent dye (e.g SYBR Green I).
- Plain PP Master Mix is especially useful for routine analysis of large sample numbers. To 0.5 ml of the Master Mix in original tube are added primers, PCR H_2O and if required DNA dye, and the Mix is stored at -20 ± 5°C. Immediately before PCR, the Mix is thawed, aliquoted and DNA templates are added.

Optimized reaction buffer

- Reaction buffer in the Plain PP Master 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 at 4°C ± 3°C (days).

Technical data

Components and packaging

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

Composition

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

Cat. No.	Product name and specification	Quantity
P201	Plain PP Master Mix (1x)	40 reactions
P202	Plain PP Master Mix (5x)	200 reactions
P203	Plain PP Master Mix (25x)	1000 reactions
P203xl	Plain PP Master Mix (100x)	4x 1000 reactions

Protocol

Suggested basic protocol for PCR amplification using Plain PP Master Mix

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

Volume*	Reagent	Final concentration
12.5 µl	Plain PP Master Mix	1x Plain PP 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, 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 PP 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 loaded into agarose gel and analyzed by electrophoresis after adding loading buffer (P048, P062, P064 or P066).

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 Plain PP Master Mix and gently homogenize.

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.