

qPCR 2x Blue Master Mix

(Cat. No. P521, P522, P523, P523xl)

rev. 04/2025

Description

This product is an alternative to qPCR 2x Master Mix with a different buffer, containing ammonium sulfate. The buffer, under certain conditions, enhances amplification, due to the optimized concentration of Mg^{2+} . The Mix is used mostly for qPCR with DNA-specific probes such as TaqMan, Molecular beacons, FRET and others.

Rapid preparation (2x concentrated)

- The qPCR 2x Blue Master Mix components are 2x concentrated (optimized reaction buffer, nucleotides, Taq DNA polymerase and anti-Taq monoclonal antibody). The samples are prepared simply by mixing an aliquot of the Mix with oligonucleotide primers, template DNA, H₂O (included) and selected DNA probes. This facilitates rapid preparation of the PCRs.

Hot start

- The product contains monoclonal antibody anti-Taq, which binds to Taq DNA polymerase and thus inactivates its enzymatic activity. After the first denaturation cycle, the antibody is irreversibly inactivated and Taq DNA polymerase regains enzymatic activity. This decreases the formation of nonspecific DNA amplicons.

Sensitive detection

- This product is optimized for the sensitive detection of DNA fragments amplified during qPCR from genomic DNA or cDNA obtained by reverse transcription.

Rapid setup

- qPCR 2x Blue Master Mix is especially useful for routine analyses of large numbers of DNA samples. To 0.5 ml of the Master Mix in the original tube, primers (e.g. 40 μ l forward and 40 μ l reverse), PCR H₂O and fluorescent probes are added and mixed; the "armed Mix" can be stored at $-20 \pm 5^\circ\text{C}$. Immediately before PCR, the Mix is thawed and each 24 μ l aliquot is mixed with 1 μ l of the tested DNA template.

Technical data

Components and packaging

- 1 tube with 0.5 ml qPCR 2x Blue Master Mix (for 40 reactions, 25 μ l each).
- 1 tube with 1.5 ml PCR H₂O.

Storage

- At temperature $-20^\circ\text{C} \pm 5^\circ\text{C}$. Material can be repeatedly defrosted.

Composition

- The Mix is 2x concentrated: 150 mM Tris-HCl, pH 8.8 (at 25°C), 40 mM $(\text{NH}_4)_2\text{SO}_4$, 0.02% Tween 20, 5 mM MgCl_2 , 400 μ M dATP, 400 μ M dCTP, 400 μ M dGTP, 400 μ M dTTP, Taq DNA polymerase (50 U/ml), monoclonal antibody anti-Taq, stabilizers and additives.

Purity and quality control

- Each batch of qPCR 2x Blue Master Mix is tested for amplification of a single copy gene in genomic DNA.

| Cat. No. | Product name and specification | Quantity |
|----------|--------------------------------|------------------|
| P521 | qPCR 2x Blue Master Mix (1x) | 40 reactions |
| P522 | qPCR 2x Blue Master Mix (5x) | 200 reactions |
| P523 | qPCR 2x Blue Master Mix (25x) | 1000 reactions |
| P523xl | qPCR 2x Blue Master Mix (100x) | 4x1000 reactions |



Protocol

Suggested protocol for PCR amplification using qPCR 2x Blue Master Mix

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

| Reagent | Volume* | Final concentration |
|--------------------------------------|---------|---|
| qPCR 2x Blue Master Mix | 12.5 µl | 75 mM Tris-HCl, pH 8.8 (25°C), 20 mM (NH ₄) ₂ SO ₄ , 0.01% Tween 20, 2.5 mM MgCl ₂ , 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 200 µM dTTP, 25 U/ml Taq DNA polymerase, monoclonal antibody anti-Taq, stabilizers and additives |
| 5' primer (50 µM) | 1 µl | 0.1 - 1 µM (~ 20 bases in length) |
| 3' primer (50 µM) | 1 µl | 0.1 - 1 µM (~ 20 bases in length) |
| Template DNA (1 ng/µl - 1 µg/µl) | 1 ul | 0.02 ng/µl – 0.02 µg/µl |
| Fluorescent DNA probe | 1 µl | |
| PCR H ₂ O (Cat. No. P042) | 8.5 ul | to a final volume 25 µl |

*Different volumes can be used, but qPCR 2x Blue Master Mix finally be diluted twice.

2. Mix gently and briefly centrifuge.

3. Perform real-time PCR under conditions optimized for the primers used.

Standard cycling parameters for ~200 bps amplicons are:

Initial denaturation step 94°C, 5 min.

40 cycles on a cycler for real-time PCR:

Further denaturation steps 94°C, 15 sec.

| | |
|-------------------|---------------|
| Primers annealing | 56°C, 15 sec. |
|-------------------|---------------|

Extension 72°C, 30 sec.