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qPCR 2x Master Mix

(Cat. No. P501, P502, P503, P503xl) rev. 04/2025

Description

qPCR 2x Master Mix has the same composition as qPCR 2x SYBR Master Mix (Cat. No. P551) except that it does not contain DNA binding dye. It is used mostly for qPCR with DNA specific probes such as TaqMan, Molecular beaqcons, FRET and others.

Rapid samples preparation

- All components of the qPCR 2x Master Mix are 2x concentrated, which facilitates rapid preparation of the PCRs. The samples are prepared by mixing an aliquot of the Mix with oligonucleotide primers, template DNA, H_2O (included) and selected DNA probes.

Hot start

- qPCR 2x Master Mix contains monoclonal antibody anti-Taq, which inactivates enzymatic activity of the enzyme. After the first denaturation cycle, the antibody is irreversibly inactivated and Taq DNA polymerase regains enzymatic activity.

Sensitive detection

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

Rapid setup

- qPCR 2x Master Mix is especially useful for routine analyses of large numbers of DNA samples. To 0.5 ml of the Master Mix in original tube, primers (e.g. 40 µl forward and 40 µl reverse) PCR H_2O and fluorescent probes are added and mixed; the "armed" Mix can be stored at -20 ± 5°C. Immediately before PCR, the Mix is thawed and each 24 µl aliquot is mixed with 1 µl of the tested DNA template.

Technical data

Components and packaging

- 1 tube with 0.5 ml qPCR 2x Master Mix (for 40 reactions, 25 µl each).
- 1 tube with 1.5 ml PCR H_2O.

Storage

- At temperature -20°C ± 5°C. Material can be repeatedly defrosted.

Composition

- The Mix is 2x concentrated: 20 mM Tris-HCl, pH 8. 8 (at 25°C), 100 mM KCl, 0.2 Triton X-100, 3 mM $MgCl_2$, 400 µM dATP, 400 µM dCTP, 400 µM dGTP, 400 µM dTTP, Taq DNA polymerase (50 U/ml), monoclonal antibody anti-Taq, stabilizers and additives.

Purity and quality control

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

Cat. No.	Product name and specification	Quantity
P501	qPCR 2x Master Mix (1x)	40 reactions
P502	qPCR 2x Master Mix (5x)	200 reactions
P503	qPCR 2x Master Mix (25x)	1000 reactions
P503xl	qPCR 2x Master Mix (100x)	4x 1000 reactions

Protocol

Suggested protocol for PCR amplification using qPCR 2x Master Mix

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

Reagent	Volume*	Final concentration
qPCR 2x Master Mix	12.5 µl	10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.1% Triton X-100, 1.5 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 (19nM), 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_2O (Cat. No. P042)	8.5 ul	to a final volume 25 µl

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

2. Mix gently and briefly centrifuge.

3. 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 Primers annealing 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.