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qPCR 2x Blue Master Mix

(Cat. No. P521, P522, P523, P523xl) rev. 02/2017

Description

This product is an alternative to qPCR 2x Master Mix with different buffer, containing ammonium sulfate. This buffer under certain conditions enhances amplification, due to 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)

- All components of the qPCR 2x Blue Master Mix are 2x concentrated (optimized reaction buffer, nucleotides, Taq DNA polymerase and anti-Taq monoclonal antibody). This facilitates rapid preparation of the PCRs. The samples are prepared simply by mixing an aliquot of the Mix with oligonucleotide primers, template DNA, H_2O (included) and selected DNA probes.

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 formation of nonspecific DNA amplicons.

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 Blue 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 Blue 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: 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, 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 $(NH_4)_2SO_4$, 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_2O (Cat. No. P042)	8.5 ul	to a final volume 25 µl

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

2. Mix gently and briefly centrifuge.

3. Perform real-time PCR under conditions optimized for the primers used.
Common cycling parameters for ~200 bps amplicons are:

Initial denaturation step 94°C, 1 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.