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qPCR 2x Blue Master Mix_BLUE

(Cat. No. B621, B622, B623, B623xl) rev. 04/2025

Description

This product is an alternative to qPCR 2x Blue Master Mix enriched with a dye qPCR Visible Blue Mark from Top-Bio (Cat. No. B129). The dye does not interfere with qPCR, but facilitates visualization of Master Mixes presence in multi-well PCR plates. 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_BLUE possesses all components 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, H_2O (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_BLUE 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 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_BLUE (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, qPCR VISIBLE Blue MARK, stabilizers and additives.

Purity and quality control

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

Cat. No.	Product name and specification	Quantity
B621	qPCR 2x Blue Master Mix_BLUE (1x)	40 reactions
B622	qPCR 2x Blue Master Mix_BLUE (5x)	200 reactions
B623	qPCR 2x Blue Master Mix_BLUE (25x)	1000 reactions
B623xl	qPCR 2x Blue Master Mix_BLUE (100x)	4x1000 reactions

Protocol

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

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

Reagent	Volume*	Final concentration
qPCR 2x Blue Master Mix_BLUE	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, qPCR Visible Blue Mark, 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_BLUE should 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:

	Temperature	Time	Number of cycles
Initial denaturaction	94°C	5 min	1
Denaturation Primers anealing Extenzion	94°C 55-65°C [1] 72°C	10 s 10 s cca 20s na 1 kb	30-45
Finální extenzion	72°C	7 min	1
Cooling	22°C		

[1] Should be determined experimentally, usually 5°C lower then melting temperature (Tm) of the primer.