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qPCR 2x SYBR Master Mix_BLUE

(Cat. No. B651, B652, B653, B653xl) rev. 11/2022

qPCR 2x SYBR Master Mix_BLUE is dedicated to qPCR quantification of DNA amplicons with fluorescent DNA dye SYBR Green I; it also contains a blue dye, which does not interfere with qPCR and facilitates visualization of Master Mixes in multi-well plates.

SYBR Green I

- The Mix contains intercalating DNA dye SYBR Green I, which after binding to double-stranded (ds)DNA, becomes strongly fluorescent with maximal excitation at 497 nm (blue light) and emission at 520 nm (green light). Because the fluorescence of unbound SYBR Green I is very low, enhanced fluorescence during qPCR corresponds to an increase in dsDNA amplicons produced during PCR.

Blue dye

- The Mix also contains a qPCR Visible Blue Mark dye from Top-Bio (Cat. No. B129) that enhances the visibility of real-time PCR reactions for more accurate pipetting, plate loading, and reaction tracking.

Hot start

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

Rapid samples preparation

- All components of the qPCR 2x SYBR Master Mix_BLUE are 2x concentrated, facilitating rapid PCR sample preparation. The samples are prepared by mixing an aliquot of the Mix with oligonucleotide primers, template DNA and H_2O (included).
- qPCR 2x SYBR Master Mix_BLUE is especially useful for routine analyses of large DNA samples. For example, to 0.5 ml of the Master Mix in the original tube, primers (e.g. 40 µl forward and 40 µl reverse) and PCR H_2O are added and mixed; the "armed" Mix can be stored at -20 ± 5°C. Immediately before use, the "armed" Mix is thawed and aliquoted into reaction wells. After addition of the DNA template, a qPCR is performed.

Technical data

Components and packaging

- 1 tube with 0.5 ml qPCR 2x SYBR Master Mix_BLUE (e.g. for 40 reactions, 25 µl each).
- 1 tube with 1.5 ml PCR Ultra H_2O.

Composition

- qPCR 2x SYBR Master Mix_BLUE contains 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, 50 U/ml Taq DNA polymerase, monoclonal antibody anti-Taq (38 nM), SYBR Green I, qPCR Visible Blue Mark, stabilizers and additives.

Storage

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

Purity and quality control

- The quality of 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 qPCR 2x SYBR Master Mix_BLUE is tested for amplification of a single copy gene in genomic DNA.

Cat. No.	Product name and specification	Quantity
B651	qPCR 2x SYBR Master Mix_BLUE (1x)	40 reactions
B652	qPCR 2x SYBR Master Mix_BLUE (5x)	200 reactions
B653	qPCR 2x SYBR Master Mix_BLUE (25x)	1000 reactions
B653xl	qPCR 2x SYBR Master Mix_BLUE (100x)	4x1000 reactions

Protocol

The suggested basic protocol for PCR amplification using qPCR 2x SYBR Master Mix_BLUE

1. In a thin-walled PCR tube or wells of the plate, the following components are mixed

Reagent	Volume*	Final concentration
qPCR 2x SYBR Master Mix_BLUE	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), SYBR Green I, 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
PCR H_2O (Cat. No. P042)	9.5 ul	to a final volume 25 µl

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

2. Mix gently and briefly centrifuge.

3. Perform real-time PCR on a qPCR cycler under conditions optimized for the primers used. Standard cycling parameters are:

I. Initial denaturation, 94°C, 5 min

II. Cycling and amplification of the template
Denaturation 94°C, 10 sec
Primers annealing 55 - 65°C (depending on the primers), 10 sec
Extension 72°C, 10-30 sec (~20 sec for 500 bps)
During this step, fluorescence of SYBR Green I is measured
35 - 45 cycles

III. High-resolution melting (HRM) analysis
Denaturation 94°C, 10 sec
Hybridization 65°C, 1 min
Continually increase the temperature from 65°C to 94°C with measurement of the fluorescence of SYBR green I.