Top-Bio s.r.o.

Tel: +420 603 476 934
E-mail: top-bio@top-bio.cz
www.top-bio.com

qPCR 2x SYBR Master Mix

(Cat. No. P551, P552, P553, P553xl) rev. 01/2022

qPCR 2x SYBR Master Mix is dedicated to qPCR with quantification of the DNA amplicons with fluorescent DNA dye SYBR Green I.

SYBR Green I

- The Mix contains fluorescent 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.

Hot start

- qPCR 2x SYBR Master 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 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 is especially useful for routine analyses of large 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 use, the Mix is thawed and each 24 µl aliquot is mixed with 1 µl of the tested DNA template and qPCR is performed.

Technical data

Components and packaging

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

Composition

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

Cat. No.	Product name and specification	Quantity
P551	qPCR 2x SYBR Master Mix (1x)	40 reactions
P552	qPCR 2x SYBR Master Mix (5x)	200 reactions
P553	qPCR 2x SYBR Master Mix (25x)	1000 reactions
P553xl	qPCR 2x SYBR Master Mix (100x)	4x1000 reactions

Protocol

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

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

Reagent	Volume*	Final concentration
qPCR 2x SYBR 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), SYBR Green I, 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 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, measure the fluorescence of SYBR Green I.
40 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 and measure the fluorescence of SYBR Green I.