Top-Bio s.r.o.

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

qPCR 2x SYTO-9 Master Mix

(Cat. No. P591, P592, P593, P593xl) rev. 04/2025

Description

qPCR 2x SYTO-9 Master Mix is dedicated for more demanding qPCRs with quantification of the DNA amplicons with fluorescent DNA dye SYTO-9. This Master Mix is especially useful for amplification of larger DNA fragments (upto 1 kbp) and for demanding high resolution melting (HRM) analysis.

SYTO-9

- The Mix contains fluorescent DNA dye SYTO-9, which after binding to double-stranded (ds)DNA becomes strongly fluorescent with maximal excitation at 485 nm and emission at 498 nm (green light). These spectral parameters are similar to those of SYBR green I (497/520 nm) and therefor the same filter set can be used. Because fluorescence of unbound SYTO-9 is very low, enhanced fluorescence corresponds to an increase in dsDNA amplicons produced during PCR.

Hot start

- qPCR 2x SYTO-9 Master Mix contains anti-Taq DNA polymerase monoclonal antibody which inactivates 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 SYTO-9 Master Mix are 2x concentrated, which facilitates rapid preparation of the PCR samples. The samples are prepared by mixing an aliquot of the Mix with oligonucleotide primers, template DNA and H_2O (included).
- qPCR 2x SYTO-9 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), fluorescent probes and PCR H_2O (upto 960 µl) are added and mixed; such "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 SYTO-9 Master Mix (for 40 reactions, 25 µl each).
- 1 tube with 1.5 ml PCR H_2O.

Composition

- qPCR 2x SYTO-9 Master Mix contains: 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, 50 U/ml Taq DNA polymerase, monoclonal antibody anti-Taq (38 nM), SYTO-9 dye, stabilizers and additives.

Storage

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

Purity and quality control

- Each batch of qPCR 2x SYTO-9 Master Mix is tested for amplification of a single copy gene in genomic DNA.

Cat. No.	Product name and specification	Quantity
P591	qPCR 2x SYTO-9 Master Mix (1x)	40 reactions
P592	qPCR 2x SYTO-9 Master Mix (5x)	200 reactions
P593	qPCR 2x SYTO-9 Master Mix (25x)	1000 reactions
P593xl	qPCR 2x SYTO-9 Master Mix (100x)	4x 1000 reactions

Protocol

Suggested basic protocol for PCR amplification using qPCR 2x SYTO-9 Master Mix

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

Reagent	Volume*	Final concentration
qPCR 2x SYTO-9 Master Mix	12,5 µl	75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2.5 mM $MgCl_2$, 200 µM každý dNTP, 25 U/ml Taq DNA polymerase, monoclonal antibody anti-Taq (19nM), DNA dye SYTO-9, 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 SYTO-9 Master Mix should be finally diluted twice.

2. Mix gently and briefly centrifuge.

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

Denaturation at 94°C, 1 min
Primers annealing at 55°C, 30 sec
Extension at 72°C, 30 sec
40 cycles on a cycler for real-time PCR