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TP SYBR 2x Master Mix

(Cat. No T605, T606, T607, T607xl) rev. 02/2022

Description

TP SYBR 2x Master Mix is dedicated for universal analysis of DNA samples using qPCR with quantification of DNA amplicons with DNA dye SYBR Green I. It is based on recent finding that addition of **T**rehalose or 1,2-**P**ropanediol (abbriated **TP**) into reaction mixture is capable of susbstantialy increasing efficiency of PCR and enhance amplification of samples which are otherwise difficult to amplify, including DNA from whole blood, GC rich amplicons and samples containing PCR inhibitors ***(Horáková a spol., BMC Biotechnology, 11:41, 2011)***.

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 fluorescence of unbound SYBR Green I is low, enhanced fluorescence during qPCR corresponds to an increase in dsDNA amplicons produced during PCR.

Rapid preparation of the samples

- All components of the TP SYBR 2x Master Mix are 2x concentrated (optimized reaction buffer containing trehalose and 1,2-propanediol, nucleotides, Taq DNA polymerase, SYBR Green I) which facilitates rapid preparation of the samples. The samples are prepared by mixing an aliquot of the Master Mix with oligonucleotide primers, template DNA and H_2O (included).
- TP SYBR 2x 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) and PCR H_2O (e.g. 380 µl) are added and mixed; the "armed" Mix can be stored at -20 ± 5°C. Immediately before use, the Mix is thawed and each e.g. 24 µl aliquot is mixed with e.g. 1 µl of the tested DNA template.

Technical data

Components and packaging

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

Storage

- For very short terms (hours, days) at room temperature
- For short terms (weeks) at 4 - 8°C.
- For long terms at -20 ± 5°C. Material can be repeatedly defrosted

Composition:

- TP SYBR 2x Master Mix contains: 150 mM Tris-HCl, pH 8.8 (25°C), 40 mM $(NH_4)_2SO_4$, 0.4 M trehalose, 2 M 1,2-propanediol, 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), SYBR Green I, stabilizers and additives.

Purity and quality control:

Each batch of TP SYBR 2x Master Mix is tested for amplification of a single copy gene with high content of GC in genomic DNA.

Cat. No.	Product name	Quantity
T605	TP SYBR 2x Master Mix (1x)	40 reactions
T606	TP SYBR 2x Master Mix (4x)	200 reactions
T607	TP SYBR 2x Master Mix (25x)	1000 reactions
T607xl	TP SYBR 2x Master Mix (100x)	4x 1000 reactions

Protocol

Suggested basic protocol for PCR amplification using TP SYBR 2x Master Mix

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

Component	PCR in 25 µl*	Final concentration
TP SYBR 2x Master Mix**	12.5 µl	75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.2 M trehalose, 1 M 1,2-propanediol, 0.01% Tween 20, 2.5 mM $MgCl_2$, 200 µM each of dNTPs, 25 U/ml Taq DNA polymerase, SYBR Green I, stabilizers and additives
5´ primer (50 µM)	1 µl	2 µM
3´ primer (50 µM)	1 µl	2 µM
Template DNA (1 ng/µl - 1 µg/µl); or nonseparated 2x diluted blood	1 ul	0.04 ng – 0.04 µg DNA/µl
PCR H_2O (Cat. No. P042)	9.5 ul	

* Different volumes can be used, but the Master Mix should be finally diluted twice.

** Before using frozen Master Mix it is important that all components are completely soluble. Solubilization is accelerated by warming Master Mix at 37°C.

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:

I. Initial denaturation, 94°C, 10 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 I is measured

Repeat 30 – 40x

III. HRM (High resolution melting) analysis

Denaturation	94°C, 10 sec
Hybridization	65°C, 1 min

Temperature is continually increased, from 65°C to 94°C and fluorescence of SYBR green I is measured.