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# TP HS DNA-free 2x SYBR Master Mix

(Cat. No T625, T626, T627) rev. 02/2022

## Description

TP HS DNA-free 2x SYBR Master Mix is dedicated for quantitative analysis of DNA samples using qPCR based on fluorescence dye SYBR Green I. The Master Mix is not contaminated with bacterial and fungal DNA and therefore is especially useful for quantification of bacterial and fungal DNA. It contains anti-Taq monoclonal antibody for hot-start (**HS**)PCR. It also contains trehalose and 1,2-propanediol (**TP**) and thus allows amplification of the samples, which are otherwise difficult to amplify, including GC rich amplicons and samples containing PCR inhibitors (Horáková et al., BMC Biotechnology, 11:41, 2011).

### Rapid preparation of the samples

- All constant components of the Master Mix are 2x concentrated (optimized reaction buffer containing trehalose and 1,2-propanediol, nucleotides, DNA-free Taq DNA polymerase, anti-Taq polymerase monoclonal antibody, and 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, tested sample containing template DNA, and PCR $H_2O$ (included).
- TP HS DNA-free 2x SYBR Master Mix contains anti-Taq DNA monoclonal antibody, which binds and inhibits activity of the Taq DNA polymerase. This allows set up of reactions at room temperature. The antibody-based hot-start does not require a separate high temperature incubation step to activate the enzyme.
- TP HS DNA-free 2x SYBR 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 $H_2O$ (380 µl) are added and mixed; the "armed" Master Mix can be stored at -20 ± 5°C. Immediately before use, the Master Mix is thawed, each 24 µl aliquot is mixed with 1 µl of the tested sample containing DNA template and qPCR is performed.

## Technical data

### Components and packaging

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

### Storage

- For short terms (days) at 2 - 8°C.
- For long terms (weeks and months) at -20 ± 5°C.

### Composition:

- TP HS DNA-free 2x SYBR 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, DNA-free Taq DNA polymerase (50 U/ml), SYBR Green I, Taq DNA polymerase-specific monoclonal antibody, stabilizers, and additives.

### Quality control:

- Each batch of TP HS DNA-free 2x SYBR Master Mix is tested for amplification of a single copy gene with high content of GC in genomic DNA and for the absence of bacterial DNA in the Master Mix; in samples with primers for bacterial DNA, but without externally added template DNA no amplicons are detected after 40 cycles of amplification.

| Cat. No. | Product name | Quantity |
|---|---|---|
| T625 | TP HS DNA-free 2x SYBR Master Mix | 40 reactions |
| T626 | TP HS DNA-free 2x SYBR Master Mix | 200 reactions |
| T627 | TP HS DNA-free 2x SYBR Master Mix | 1000 reactions |

## Protocol

When analysing bacterial or fungal DNA it is necessary to work under **sterile** conditions.
Suggested basic protocol for qPCR amplification using TP HS DNA-free 2x SYBR Master Mix.

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

| **Component** | **PCR in 25 µl*** | **Final concentration** |
|---|---|---|
| TP HS DNA-free 2x SYBR Master Mix** | 12.5 µl | 75 mM Tris-HCl, pH 8.8 (25°C), 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 dNTP, 25 U/ml DNA-free Taq DNA polymerase, Taq DNA polymerase-specific monoclonal antibody, 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 whole 2x diluted blood | 1 µl | 0.04 ng – 0.04 µg DNA/µl |
| PCR Ultra $H_2O$ (Cat. No. P040) | 9.5 µl | |

* Different volumes can be used, but the TP HS DNA-free 2x SYBR 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 short warming of the Master Mix to 37°C and mixing by vortexing.

2. Mix gently by vortexing and briefly centrifuge.

3. Perform qPCR under conditions optimized for the primers used. Common cycling parameters are:

| | **Temperature** | **Time** | **Number of cycles** |
|---|---|---|---|
| Initial denaturation | 94°C | 2 min | 1 |
| Denaturation<br>Annealing of primers<br>Extension | 94°C<br>55-65°C<br>72°C | 15 s<br>15 s<br>1 min per 1 kb | 25-35 |
| Final extension | 72°C | 7 min | 1 |
| Cooling | 22°C | | |

3. Amplified DNA can be loaded into agarose gel after adding a loading buffer (Cat. No P048, P064 or P066).