Control PCR*

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For the DNA Isolation of the samples see 'Automated Microbial DNA Isolation' of the **MolYsis-SN*plus*™ IVD** manual.

The ingredients of this kit do not require Material Safety Data Sheets (MSDS's).

Explanation of the PCR working place:

Work in a PCR cabinet. Take care that all handling is performed in a DNA- and DNase-free environment.

Assay *Control PCR*:

- 1 reaction per sample
- 1 reaction for the positive PCR control (PC, *Control DNA (1x)*)
- 1 reaction for negative PCR control (NC, H_2O)

Before starting the mastermix preparation:

Thaw the following vials at room temperature (+18 to +25°C):

- H_2O
- *MA Control* (2.5x)
- *DS* (light sensitive)
- *Control DNA (50x)*
- *Dilution buffer*

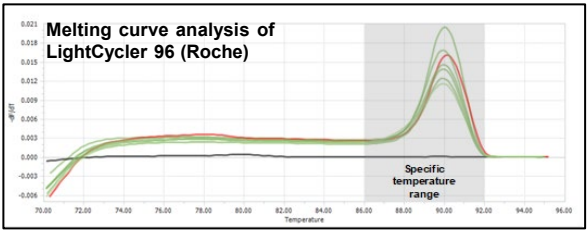
Vortex thawed PCR reagent vials for a few seconds to mix and briefly centrifuge to clear the lid.

Preparation of Positive PCR Control (PC, *Control DNA (1x)*):

1. Vortex *Control DNA (50x)* and *Dilution buffer* vials and pulse centrifuge.
2. Pipette **245 µl** *Dilution buffer* in a 1.5 ml tube (DNA- and DNase-free; not supplied)
3. Add **5 µl** *Control DNA (50x)* and vortex to mix.
4. Briefly centrifuge to clear lid.
5. Label dilution with *Control DNA (1x)* and the preparation date (PC to be used for 2 months; store at +4 to +12°C).

PCR Assaying

The *assay Control PCR* shows a specific signal (peak) in the melting curve analysis for positive PCR control (red curve) and no signal for the negative PCR control (black curve). All samples (green curves) show a valid result.



Interpretation of PCR Results

Please note:** **Before using this scheme inform yourself of the details of the procedure.** Special care is required for working under DNA- and DNase-free conditions. **Please consult the manual for more information.

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Note: Keep all tubes filled with **mastermix** and the ***MolTaq 16S/18S*** chilled in a cooling rack **(-15 to -25°C)**. Do not interrupt the cooling. ***Cooling*** of the tubes ***is important*** to minimize the generation of primer dimers.

1. **Arrange PCR tubes** (not supplied) for mastermix in a cooling rack and mark.
2. **Briefly** centrifuge *MolTaq 16S/18S* and place it in the cooling rack (-15 to -25°C).
3. **Place a 1.5 ml tube** (not supplied) in a cooling rack. **Pipette the following components** of the assay Control PCR into the tube (see 'PCR Assaying' page 1, short manual). Preparation of mastermix see Tab. 1. Vortex tube to mix and briefly centrifuge.

Table 1: Preparation of mastermix (assay Control PCR). Volumes in µl.

Reactions	*MA Control*	*H_2O*	*DS*	*MolTaq 16S/18S*
1	10.0	7.5	2.5	0.8
2	20.0	15.0	5.0	1.6
3	30.0	22.5	7.5	2.4
4	40.0	30.0	10.0	3.2
5	50.0	37.5	12.5	1.0
6	60.0	45.0	15.0	4.8
7	70.0	52.5	17.5	5.6
8	80.0	60.0	20.0	6.4
9	90.0	67.5	22.5	7.2
10	100.0	75.0	25.0	8.0

Preparation of mastermix

4. **Pipette 20 µl** of mastermix into the chilled (-15 to -25°C) PCR tubes per reaction (dedicated for samples, PC and NC, respectively).
5. **Add 5 µl** *H_2O* as PCR negative control (NC).
6. **Add 5 µl** of each sample eluate.
7. **Add 5 µl** *Control DNA (1x)* as PCR positive control (PC)
8. **Close** PCR tubes with caps.
9. **Continue with section 'PCR Thermocycling'** of manual.
   Interpretation of the PCR results see on page 1 of the short manual.

Please note:** **Before using this scheme inform yourself of the details of the procedure.** Special care is required for working under DNA- and DNase-free conditions and secure working conditions. **Please consult the manual for more information.

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