Mastermix 16S Complete, DNA-free

For the PCR detection and identification of bacteria using universal 16S rDNA primers

For research use only

Cat. No. S-020-0100	**100 reactions**
Cat. No. S-020-0250	**250 reactions**
Cat. No. S-020-1000	**1000 reactions**

Product overview

Kit/Component

Mastermix 16S Complete			
	100 rxn	**250 rxn**	**1000 rxn**
2.5 x mastermix (3 mM $MgCl_2$ final concentration), incl. primers	2 x 0.5 ml	5 x 0.5 ml	20 x 0.5 ml
MolTaq 16S DNA polymerase (non-Hot Start)	0.08 ml	0.2 ml	4 x 0.2 ml
DNA-free PCR-grade water	1.7 ml	3 x 1.7 ml	12 x 1.7 ml
DNA staining solution, 10x concentrated	0.25 ml	0.625 ml	4 x 0.625 ml
Gel loading solution, 6x concentrated	0.2 ml	0.5 ml	4 x 0.5 ml
DNA size marker (1 kb), pre-stained	0.05 ml	0.125 ml	4 x 0.125 ml

Product description

Mastermix 16S Complete contains validated primers binding to conserved regions of the 16S rRNA gene. The mastermix is suitable to amplify any eubacterial DNA and thereby detect the presence of bacteria in a sample. Detection of amplified DNA is done by gel electrophoresis, using components supplied with this kit (SYBR® Green 1 DNA staining solution for visualisation, DNA marker for size estimation of the amplicon, gel loading solution). Mastermix 16S Complete may also be used for detection by Real-Time PCR with intercalating fluorescent dyes or probes or by array technologies. The amplified region (approx. 450 bp) contains variable sequences for the identification of bacteria by taxon specific probing or sequence analysis. Mastermix 16S Complete is a 2.5x-concentrated solution, the maximum final volume of the reaction mixture being 25 µl. The product contains all components necessary for a PCR run. Only supplied MolTaq 16S, DNA-free water and the template have to be added to obtain a complete reaction mixture for PCR.

Stability

Stable at -15 to -25°C for 12 months.

Applications

Detection and identification of eubacteria by amplification of a region of the 16S rRNA gene.

Packaging, Storage and Handling

The purification of the mastermix and its confectioning are done under standard precautions for the avoidance of air-borne and handling-based DNA contaminations. The mastermix is supplied as a 2.5x-concentrated solution in DNA-free screw cap vials. Store all vials in the kit at -15 °C to -25 °C upon receipt. For usage, the mastermix and the other components of the kit are thawed at room temperature (18 to 25 °C) and stored at 4 °C. After removal of solution for use, freeze again for storage (-15 to -25 °C). It is important to note that the DNA staining solution is sensitive to light and should be stored in the dark also during handling and use. Take care to maintain a DNA-free environment during opening the vials and handling the mastermix. Use only certified bacterial DNA-free pipette tips and PCR consumables for running the assay. Please contact Molzym for further information regarding our products and other suppliers of DNA-free plastic consumables.

Quality control and specifications

Negative PCR controls using DNA-free water instead of template DNA are used for analysis of contamination of bacterial DNA in the purified final mastermix. Guarantee is given for the absence of signals in negative controls at a rate of ≥ 97% for up to 40 PCR cycles (provided the avoidance of contamination by handling errors). DNA-free mastermix is defined as giving no bacterial DNA-specific signal. In negative control runs, the absence of banding in gel electrophoretic analysis must be demonstrated. Positive controls are run using known amounts of genomic DNA extracted and purified by PrestoSpin D Bug kit (Molzym GmbH & Co.KG,

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cat.# D-040-050) from *Staphylococcus aureus* or other bacteria. Alternatively, use Molzym's DNA positive control (cat. no. S-200-050).

Patents/Disclaimer
Some applications, in particular Real-Time PCR, in which this product may be used are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used. Patents especially to be mentioned are those for Real-Time PCR and the use of intercalating fluorescent dyes and probes: EP 543942, EP 919565, US 5210015, US 5487972, US 5804375, US 6214979, EP 512 334, US 5994056, US 6171785, US 5538848, US 5723591, US 5952202, US 5876930, US 6030787, US 6258569, US 6821727.

Tradenames
Opticon® (BioRad), LightCycler® (Hoffmann LaRoche), StepOne® (Applied Biosystems), SYBR® Green 1 (Invitrogen).

PCR protocol

Take care that all handling is done in a DNA-free environment (UV-irradiated workstation). Make sure that plastic consumables (including PCR vials, pipette tips, screw cap polypropylene tubes) are free of contaminating bacterial DNA when used in combination with the amplification reaction mixture. Work according to the sequence of steps below:

1. **Thaw mastermix at room temperature (18 to 25 °C). Vortex for a few seconds to mix and briefly centrifuge vial. Store at 4 °C for further use. After use, store at -15 to -25 °C.**
2. **Pipette x µl DNA-free water (for a volume of 25 µl) into each PCR vial. Keep vials chilled.**
3. **Add 10 µl of the 2.5x mastermix**
4. **Add 0.8 µl MolTaq 16S**
5. **Finally add the template. Seal vials and keep chilled until placing in a PCR machine.**
6. **Start the programme of the assay (see below).**

Pipetting scheme:

- **x µl DNA-free water (supplied)**
- **10 µl 2.5x mastermix**
- **0.8 µl MolTaq 16S**
- **y µl template**

25.8 µl in total

Vortex for 5 sec and pipette your prepared mastermix to each PCR vial and add the template DNA to a final volume of 25 µl. With each series of PCR, run a positive control comprising a DNA standard (0.5 to 5 ng per reaction) extracted from a bacterial culture (e.g. Molzym's DNA positive control; cat. no. S-200-050).

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PCR thermocycling conditions: Eppendorf Mastercycler Gradient

Initial Denaturation: 95°C for 1 min,

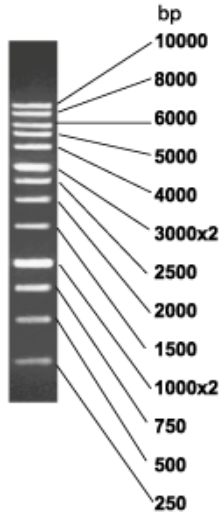
Cycling: 40 cycles of 95 °C for 5 s, 55 °C for 5 s, 72 °C for 25 s

Other cyclers have to be validated for thermocycling using Mastermix 16S Complete. For this, use Molzym's DNA positive control (cat. no. S-200-050).

Detection by agarose gel electrophoresis:
The DNA staining solution supplied with this kit is designed for gel electrophoretic analysis. Thaw the solution at room temperature (18 to 25 °C), store at 4 °C and make sure that it is kept in the dark. Do not freeze again and store at 4 °C. Prepare a 2 % (w/v) agarose gel in 1x TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.3) in a 250 ml Erlenmeyer flask by heating in a microwave oven until boiling. When the agarose is dissolved, cool down the solution to approx. 50°C. Pour into a suitable gel tray engaged with rubber gaskets and supplied with a comb in the notches of the gel tray. Once the gel is solid (after approx. 30 min), remove the gel tray from the electrophoresis chamber, remove the comb and put the tray back into the chamber filled with 1x TAE buffer. The gel should be covered with 1 to 2 cm of buffer.

For analysis, mix 9 µl of the PCR solution containing the amplicon with 1 µl of the DNA staining solution and 2 µl of the gel loading solution in an Eppendorf tube or in a well of a 96 well plate. Let stand in the dark for 15 min to bind the stain to the amplicon DNA. Pipette the mixture (12 µl) into an indentation of the gel. Into one lane alongside those containing your samples load 5 µl of the DNA size marker. Cover the electrophoresis chamber with the plastic cover and run the gel at the recommended maximum voltage setting of the system (e .g. 5 V/cm gel) in the dark. Leave the gel running until the fastest blue dye has moved about 2/3 of the way through the gel.

Remove the gel, place it under a UV lamp or on a transilluminator and photograph. Compare potentially appearing bands with the DNA size marker and positive control, which has a size of approx. 450 bp.



DNA size marker (1kb ladder) as a reference for gel electrophoretic detection of bacterial amplicons

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Interpretation of the results

Gelelectrophoresis:

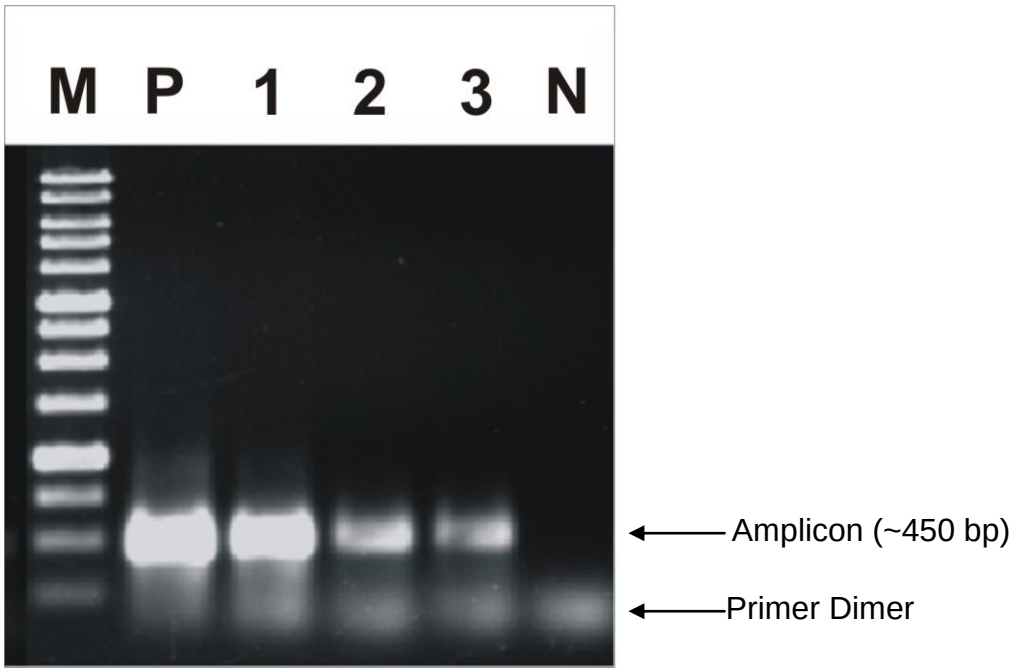

Fig. 1:

The size of the amplicon should be around 450bp (upper arrow). The lower arrow shows primer dimers which may appear even in the negative control. P: Positive control, 1-3: samples, N: Negative Control

Identification of bacteria by sequencing:

Sequence identification of the amplicon produced with the Mastermix 16S Complete can be performed using a primer for gram positive bacteria (SeqGP16S) and one for gram negative bacteria (SeqGN16S). A set of these primers can be ordered at Molzym (cat. no. S-775-100).

For sequencing of amplicons the PCR reaction needs to be purified by a commercial PCR purification kit. For this purpose, use the remaining aliquot of the PCR reaction mixture (16 µl) and follow the instructions of the manufacturer of the kit. Elute the amplicon from the column using sterile deionised water. The procedure may not take more than 15 min. Apply the eluted DNA to a sequencing reaction as advised by the manufacturer of the sequencing system.

For identification of the detected bacteria, perform an online search with the nucleotide sequence obtained. For guidance, see e. g. Sepsitest-BLAST (http://www.sepsitest-blast.de/).

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Addendum

Real-Time PCR Protocols

Using Mastermix 16S Complete for Real-Time PCR (DNA staining solution)
Mastermix 16S Complete can easily be used for the detection of bacterial DNA by Real-Time PCR. The reaction mixture of Mastermix 16S Complete has simply to be supplemented by the DNA staining solution supplied with this product.

Protocol A

Real-Time PCR with PCR vials and strips (qPCR machines, e.g. BioRad Opticon®, ABI StepOne®)

Take care that all handling is done in a DNA-free environment (UV irradiated workstation). Make sure that plastic consumables (including PCR vials, pipette tips, screw cap polypropylene tubes) are free of contaminating bacterial DNA when used in combination with the amplification reaction mixture. Work according to the sequence of steps below.

1. **Thaw mastermix at room temperature, then store at 4 °C. Vortex for a few seconds to mix and briefly centrifuge the vial. Thaw DNA staining solution at room temperature, then store at 4 °C, briefly centrifuge and keep in the dark until use.**
2. **Pipette x µl DNA-free water (for a volume of 25 µl) into each PCR vial. Keep vials chilled.**
3. **Add 10 µl of the 2.5x mastermix**
4. **Add 2.5 µl of the 10x DNA staining solution**
5. **Add 0.8 µl MolTaq 16S**
6. **Finally add the template. Seal vials and keep chilled until placing in a PCR machine**
7. **Start the programme of the assay (see below)**

Vortex for 5 s and pipette your prepared mastermix to each PCR vial and add the template DNA to a final volume of 25 µl. With each series of Real-Time PCR, run a positive control comprising a DNA standard (0.5 to 5 ng per reaction) extracted from a bacterial culture (e.g. Molzym's DNA positive control; cat. no. S-200-050). Make sure that the vials are kept dark until placing into the Real-Time PCR machine.

Real-Time PCR thermocycling conditions: For Real-Time PCR machines from Applied Biosystems, switch off the internal reference ROX before the PCR run! Set to the appropriate channel for SYBR Green 1 detection.

Initial Denaturation: 95°C for 1 min.

Cycling: 40 cycles of 95°C for 5 s, 55°C for 5 s, 72°C for 25 s

T_m Analysis: 70 to 95°C, read every 0.2°C, hold for 1 s between reads

Other Real-Time PCR cyclers have to be validated for thermocycling using Mastermix 16S Complete. For this, use Molzym's DNA positive control (cat. no. S-200-050).

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Protocol B

Real-Time PCR with 20 µl glass capillaries (Roche LightCycler® 1.5 and 2.0)
Take care that all handling is done in a DNA-free environment (UV irradiated workstation). Make sure that plastic consumables (including PCR vials, pipette tips, screw cap polypropylene tubes) are free of contaminating bacterial DNA when used in combination with the amplification reaction mixture. Work according to the sequence of steps below. This protocol is designed for 20 µl assay volume which leaves 25% of the supplied mastermix. For further use please order MolTaq 16S (cat. no. P-019-100).

1. **Thaw mastermix at room temperature, then store at 4 °C. Vortex for a few seconds to mix and briefly centrifuge the vial. Thaw DNA staining solution at room temperature, then store at 4 °C, briefly centrifuge and keep in the dark until use.**
2. **Pipette x µl DNA-free water (for a volume of 20 µl) into each LightCycler® capillary. Keep capillaries chilled**
3. **Add 8 µl of the 2.5x mastermix**
4. **Add 2 µl of the 10x DNA staining solution**
5. **Add 0.8 µl MolTaq 16S**
6. **Finally add the template or supplied DNA-free water (negative control). Seal capillaries, centrifuge according to the instructions of the manufacturer and keep chilled in the dark until placing in the Real-Time PCR machine. Start the specific programme of the assay (see below)**

Vortex for 5 sec and pipette your prepared mastermix to each PCR vial and add the template DNA to a final volume of 20 µl, seal capillaries and centrifuge. With each series of Real-Time PCR, run a positive control comprising a DNA standard (0.5 to 5 ng per reaction) extracted from a bacterial culture. Make sure that the vials are kept dark until placing into the Real-Time PCR machine.

Real-Time PCR thermocycling conditions:

Initial Denaturation: 95°C for 1 min,

Cycling: 40 cycles of 95°C for 1 s, 55°C for 5 s, 72°C for 25 s

T_m Analysis: from 65°C to 95°C, 0.05°C temperature transition rate

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Interpretation of the results:

Real Time PCR (Light Cycler, Roche):

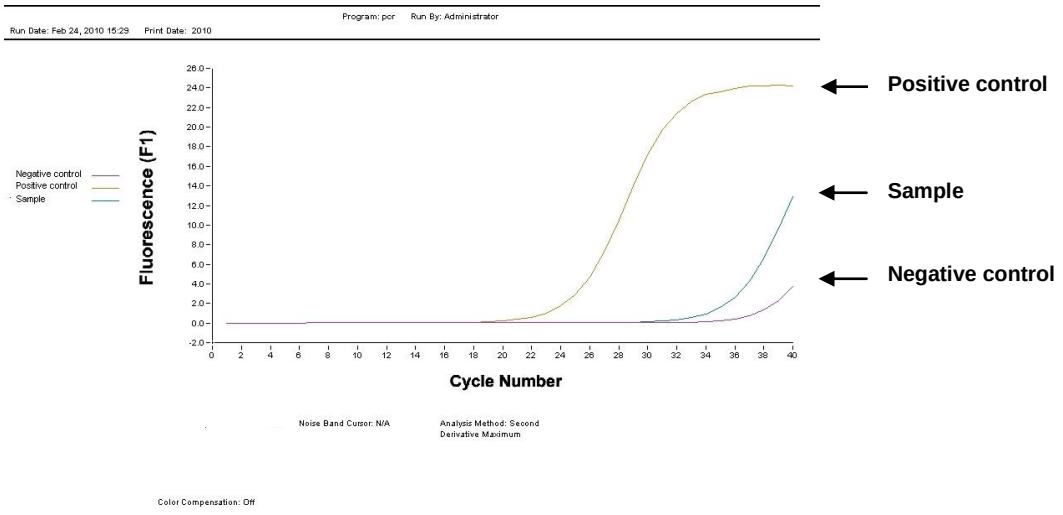

Fig. 2:

Fluorescence curve: Please note that also the negative control may produce a signal due to primer dimer formation. It is always necessary to run a melting curve analysis (see fig. 3) in order to distinguish between primer dimers and the specific amplicon.

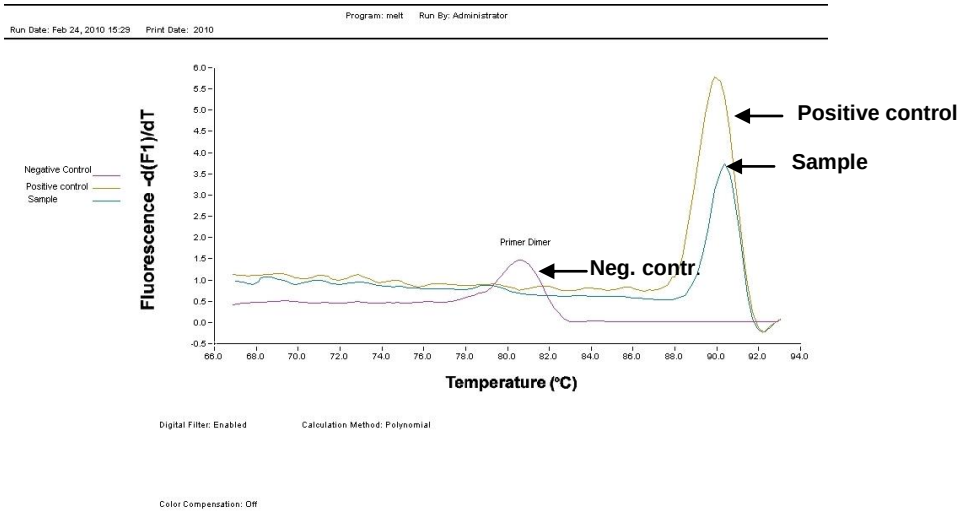

Fig. 3:

Melting curve analysis from the Real time run above (fig. 2). Note that the primer dimers in the negative control show a peak around 80°C, whereas a specific amplicon shows a peak around 87°C (see Pos. control and sample).

Identification

Follow the instructions given on page 4 (Identification of bacteria by sequencing).

Please address any questions relating the mastermix to the support hotline:

Email: support@molzym.com / Tel.: +49(0)421-696162-0

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