

## Mastermix 16S Dye, DNA-free

For the PCR detection and identification of bacterial or fungal DNA targets with custom primers

For research use only

Cat. No. S-030-0100	100 reactions
Cat. No. S-030-0250	250 reactions
Cat. No. S-030-1000	1000 reactions

### Product overview

#### Kit/Component

Mastermix 16S Dye	100 rxn	250 rxn	1000 rxn
2.5 x mastermix (3 mM Mg <sup>2+</sup> final concentration)	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	10 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	2 ml
DNA size marker (1 kb ladder), pre-stained	0.05 ml	0.125 ml	0.5 ml

### Product description

Mastermix 16S Dye is designed to run PCR reactions with custom primers aimed to detect bacterial or fungal targets. The mastermix is suitable to amplify microbial DNA with added unspecific or specific primers and thereby detect the presence of bacteria or fungi 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 Dye may also be used for detection by Real-Time PCR with intercalating fluorescent dyes or probes or by array technologies. The amplicon may be used for the identification of microorganisms by taxon specific probing or sequence analysis. Mastermix 16S Dye 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. For PCR runs, supplied MolTaq 16S, DNA-free water, custom primers and/or probes and the template have to be added to obtain a complete reaction mixture.

### Stability

Stable at -15 to -25°C for 12 months.

### Applications

PCR detection and identification of bacteria and fungi by custom-specific primers

### 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. Custom primers, probes and dilution water may contain contaminating bacterial DNA that will give false positive PCR results. Take care to use only bacterial DNA-free primers, probes and dilution water with Mastermix 16S Dye.**

Please contact Molzylm 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  $\geq 97\%$  for up to 40 PCR cycles using universal 16S rDNA primers with an amplification size  $> 200$  bp (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, catalogue number 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.

## 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. Place MolTaq 16S in another cooling rack (-15 to -25 °C). After use, store components at -15 to -25 °C.**
2. **Pipette x µl of supplied 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 0.5 µl of forward primer (10 µM)**
5. **Add 0.5 µl of reverse primer (10 µM)**
6. **Add 0.8 µl MolTaq 16S**
7. **Finally add y µl of the template. Seal vials and keep chilled until placing in a PCR machine.**
8. **Start the programme of the specific assay.**

For e.g. 10 reactions prepare a 1x mastermix in a DNA-free screw cap or polypropylene vial using the following pipetting scheme (for addition of 2 µl template DNA):

- **120 µl DNA-free water**
- **100 µl 2.5x mastermix**
- **5 µl forward primer (10 µM)**
- **5 µl reverse primer (10 µM)**
- **8 µl MolTaq 16S**

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**238 µl in total**

Pipette 23 µl from this 1x mastermix to each PCR vial and add 2 µl of the template DNA or 2 µl of supplied DNA-free water (negative PCR control). With each series of PCR, run a positive control comprising a DNA standard (10 to 100 ng per reaction) extracted from a bacterial culture.

### PCR thermocycling conditions:

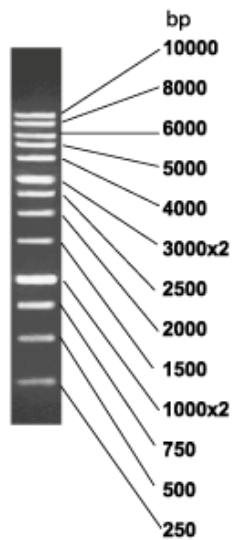
Use the specific conditions of your assay. The 1x mastermix can be used for up to 40 cycles.

### 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 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. Add 2 µl of the gel loading solution and 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.



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

**Identification of microorganisms by sequencing:**

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. <http://www.ncbi.nlm.nih.gov/>

## **Addendum**

### **Real-Time PCR Protocols**

#### **Using Mastermix 16S Dye for Real-Time PCR (DNA staining solution)**

Mastermix 16S Dye can easily be used for the detection of bacterial or fungal DNA by Real-Time PCR. The reaction mixture of Mastermix 16S Dye has simply to be supplemented by custom primers and 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 0.5 µl of forward primer (10 µM)**
- 5. Add 0.5 µl of reverse primer (10 µM)**
- 6. Add 2.5 µl of the 10x DNA staining solution**
- 7. Add 0.8 µl MolTaq 16S**
- 8. Finally add the template. Seal vials and keep chilled until placing in a PCR machine**
- 9. Start the programme of the specific assay**

For e.g. 10 reactions prepare a 1x mastermix in a DNA-free screw cap or polypropylene vial using the following pipetting scheme (for addition of 2 µl template DNA):

- **95 µl DNA-free water**
- **100 µl 2.5x mastermix**
- **5 µl forward primer (10 µM)**
- **5 µl reverse primer (10 µM)**
- **25 µl 10x DNA staining solution**
- **8 µl MolTaq 16S**

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**238 µl in total**

Vortex for 5 s and pipette 23 µl from this 1x mastermix to each PCR vial. Add 2 µl of the template DNA or 2 µl of supplied DNA-free water (negative PCR control). 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.

Use the specific conditions of your assay. The 1x mastermix can be used for up to 40 cycles.

**T<sub>m</sub> Analysis:** 70 to 95°C, read every 0.2°C, hold for 1 s between reads

## Identification

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

## 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 0.5 µl of forward primer (10 µM)**
5. **Add 0.5 µl of reverse primer (10 µM)**
6. **Add 2 µl of the 10x DNA staining solution**
7. **Add 0.8 µl MolTaq 16S**
8. **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 programme of the specific assay**

For multiples of reactions prepare a 1x mastermix in a sterile, DNA-free screw cap vial following the the pipetting scheme below as an example for 10 reactions (2 µl template DNA):

- 70 µl DNA-free water
- 80 µl 2.5x mastermix
- 5 µl forward primer (10 µM)
- 5 µl reverse primer (10 µM)
- 20 µl 10x DNA staining solution
- 8 µl MolTaq 16S

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**188 µl in total**

Vortex for 5 s and pipette 18 µl from this 1x mastermix to each capillary. Add 2 µl of the template DNA or 2 µl of supplied DNA-free water (negative PCR control), 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 (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:**

Use the specific conditions of your assay. The 1x mastermix can be used for up to 40 cycles.

**T<sub>m</sub> Analysis:** from 65°C to 95°C, 0.05°C temperature transition rate

**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](mailto:support@molzym.com) / Tel.: +49(0)421-69 61 62-0**