Mastermix 16S Primer, DNA-free

For the PCR amplification of bacterial DNA targets using universal 16S rDNA primers

For research use only

Cat. No. S-021-0100	**100 reactions**
Cat. No. S-021-0250	**250 reactions**
Cat. No. S-021-1000	**1000 reactions**

Product overview

Kit/Component

Mastermix 16S Primer			
	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 17 ml

Product description

Mastermix 16S Primer contains validated primers binding to conserved regions of the 16S rRNA gene. The mastermix is suitable to amplify any eubacterial DNA. The amplified region (approx. 450 bp) contains variable sequences for the identification of bacteria by taxon specific probing or sequence analysis. Mastermix 16S Primer is a 2.5x-concentrated solution, the 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 24 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 on ice and, after removal of aliquots for use, frozen again for storage. 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, catalogue number D-040-050) from *Staphylococcus aureus* or other bacteria.

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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 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).**

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):

- **130 µl DNA-free water**
- **100 µl mastermix**
- **8 µl MolTaq 16S**

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: 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 Primer. For this, use Molzym's DNA positive control (cat. no. S-200-050).

Use standard gel electrophoretic techniques or hybridisation probing for analysis of the PCR reaction.

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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/).

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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