Mastermix 16S Basic, DNA-free

For the PCR amplification of bacterial and fungal DNA using custom primers

For research use only

Cat. No. S-040-0100	**100 reactions**
Cat. No. S-040-0250	**250 reactions**
Cat. No. S-040-1000	**1000 reactions**

Product overview

Kit/Component

Mastermix 16S Basic			
	100 rxn	**250 rxn**	**1000 rxn**
2.5 x mastermix (3 mM Mg^{2+} 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

Product description

Mastermix 16S Basic is suitable to amplify bacterial and fungal DNA. Mastermix 16S Basic 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. 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 for 24 months from the date of manufacturing under proper storage condition. Guarantee for full performance of reagents and buffers is given through the expiration date printed on the label at the outer box, if the packed material is undamaged upon arrival and the reagents are unopened.

Applications

Detection and identification of bacteria and fungi by PCR amplification

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. 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 Basic.**
Please contact Molzym for further information regarding our products and other suppliers of DNA-free plastic consumables.

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

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**

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.

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

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

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

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