Mastermix 18S Complete, DNA-free

For the PCR detection and identification of yeasts and other fungi using universal 18S rDNA primers

For research use only

Cat. No. S-070-0100	**100 reactions**
Cat. No. S-070-0250	**250 reactions**
Cat. No. S-070-1000	**1000 reactions**

Product overview

Kit/Component

Mastermix 18S Complete			
	100 rxn	**250 rxn**	**1000 rxn**
2.5 x Mastermix 18S Yeasts (3 mM $MgCl_2$ final concentration), incl. primers	2 x 0.5ml	5 x 0.5ml	20 x 0.5ml
MolTaq 16S/18S DNA polymerase (non-Hot Start)	0.08ml	0.2ml	4 x 0.2ml
DNA-free PCR-grade water	1.7ml	3 x 1.7ml	12 x 1.7ml
DNA staining solution, 10x concentrated	0.25ml	0.625ml	4 x 0.625ml
Gel loading solution, 6x concentrated	0.2ml	0.5ml	4 x 0.5ml
DNA size marker (1 kb), pre-stained	0.05ml	0.125ml	4 x 0.125ml

Product description

Mastermix 18S Complete contains validated primers binding to conserved regions of the fungal 18S rRNA gene. The mastermix is suitable to amplify any fungal DNA and thereby detect the presence of yeasts and other 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 18S 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. 310bp) contains variable sequences for the identification of yeasts and other fungi by taxon specific probing or sequence analysis. Mastermix 18S Complete is a 2.5x-concentrated solution, the maximum final volume of the reaction mixture being 25µl. The product contains all components necessary for a PCR run. Only supplied MolTaq 16S/18S, DNA-free water and the template have to be added to obtain a complete reaction mixture for PCR.

Stability

Stable for 12 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 yeasts and other fungi by amplification of the V8/V9 variable region of the 18S 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 to +12°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 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.

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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 fungal 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 fungal 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 *Saccharomyces cerevisiae, Candida albicans* or other yeasts or fungi. 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® (Roche), 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 fungal DNA when used in combination with the amplification reaction mixture. Work according to the sequence of steps below:

1. **Thaw Mastermix 18S Yeasts at room temperature (+18 to +25 °C). Vortex for a few seconds to mix and briefly centrifuge vial. Store at +4 to +12°C for further use. After use, store at -15 to -25 °C.**
2. **Pipette x µl DNA-free water (for a volume of 25µl) into each PCR vial. Keep vials chilled.**
3. **Add 10µl of the 2.5x Mastermix 18S Yeasts**
4. **Add 0.8µl MolTaq 16S/18S**
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)**
- **10µl 2.5x Mastermix 18S Yeasts**
- **0.8µl MolTaq 16S/18S**
- **y µl template**

25.8µl 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 25µl. With each series of PCR, run a positive control comprising a DNA standard (0.5 to 5ng per reaction) extracted from a fungal 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 1min,

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

Other cyclers have to be validated for thermocycling using Mastermix 18S 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 to +12°C and make sure that it is kept in the dark. Do not freeze again and store at +4 to +12°C. Prepare a 2 % (w/v) agarose gel in 1x TAE buffer (40mM Tris-acetate, 1mM EDTA, pH 8.3) in a 250ml 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. 30min), 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 9µl of the PCR solution containing the amplicon with 1µl of the DNA staining solution and 2µl of the gel loading solution in an Eppendorf tube or in a well of a 96 well plate. Let stand in the dark for 15min to bind the stain to the amplicon DNA. Pipette the mixture (12µl) into an indentation of the gel. Into one lane alongside those containing your samples load 5µl 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. 310bp.

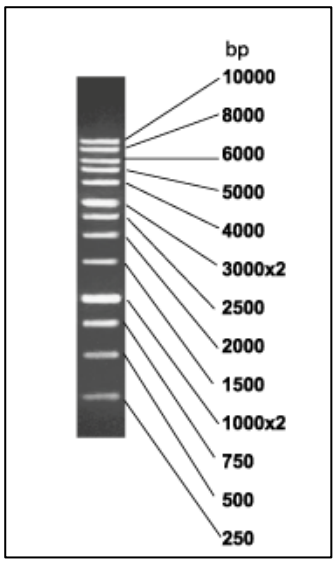

Fig. 1: DNA size marker (1kb ladder) as a reference for gel electrophoretic detection of fungal amplicons.

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Interpretation of the results

Gelelectrophoresis:

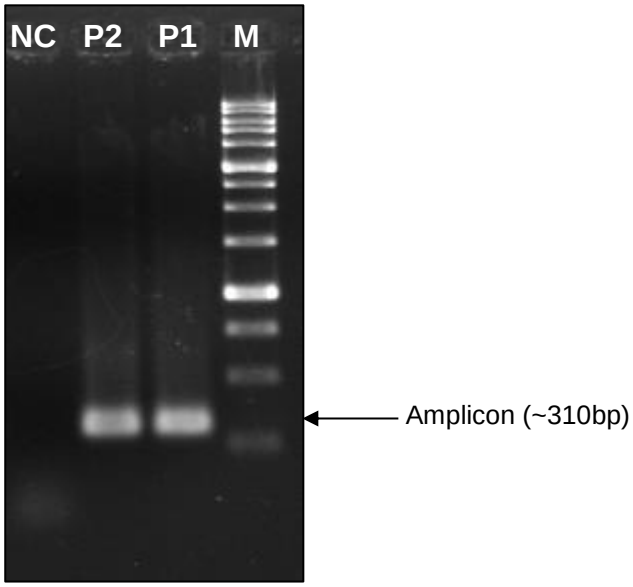

Fig. 2: PCR analysis of PCR controls using *Mastermix 18S Complete*. P1, P2: Positive PCR controls *Saccharomyces cerevisiae*; NC: Negative PCR control; M: DNA size marker (1kb ladder).
The size of the amplicon should be around 310bp. Please note: primer dimer formation may appear especially at low amounts of target DNA or even in the negative control.

Identification of yeasts and other fungi by sequencing:

Sequence identification of the amplicon produced with the Mastermix 18S Complete can be performed using a panfungal primer (SeqYeast18). The sequencing primer panfungal can be ordered at Molzym (cat. no. S-785-100).

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 (16µl) 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 15min. Apply the eluted DNA to a sequencing reaction as advised by the manufacturer of the sequencing system.

For identification of the detected fungi, 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 18S Complete for Real-Time PCR (DNA staining solution)
Mastermix 18S Complete can easily be used for the detection of fungal DNA by Real-Time PCR. The reaction mixture of Mastermix 18S 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 fungal DNA when used in combination with the amplification reaction mixture. Work according to the sequence of steps below.

1. **Thaw Mastermix 18S Yeasts at room temperature, then store at +4 to +12°C. Vortex for a few seconds to mix and briefly centrifuge the vial. Thaw DNA staining solution at room temperature, then store at +4 to +12°C, briefly centrifuge and keep in the dark until use.**
2. **Pipette x µl DNA-free water (for a volume of 25µl) into each PCR vial. Keep vials chilled.**
3. **Add 10µl of the 2.5x Mastermix 18S Yeasts**
4. **Add 2.5µl of the 10x DNA staining solution**
5. **Add 0.8µl MolTaq 16S/18S**
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 5s and pipette your prepared mastermix to each PCR vial and add the template DNA to a final volume of 25µl. With each series of Real-Time PCR, run a positive control comprising a DNA standard (0.5 to 5ng per reaction) extracted from a culture (e.g. from *Saccharomyces cerevisiae*). Alternatively use 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 1min.

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

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

Other Real-Time PCR cyclers have to be validated for thermocycling using Mastermix 18S Complete. For this, use Molzym's DNA positive control (cat. no. S-200-050).

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Protocol B

Real-Time PCR with 20µl 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 fungal DNA when used in combination with the amplification reaction mixture. Work according to the sequence of steps below. This protocol is designed for 20µl assay volume which leaves 25% of the supplied mastermix. For further use please order MolTaq 16S/18S (cat. no. P-019-100).

1. **Thaw Mastermix 18S Yeasts at room temperature, then store at +4 to +12°C. Vortex for a few seconds to mix and briefly centrifuge the vial. Thaw DNA staining solution at room temperature, then store at +4 to +12°C, briefly centrifuge and keep in the dark until use.**
2. **Pipette x µl DNA-free water (for a volume of 20µl) into each LightCycler® capillary. Keep capillaries chilled**
3. **Add 8µl of the 2.5x Mastermix 18S Yeasts**
4. **Add 2µl of the 10x DNA staining solution**
5. **Add 0.8µl MolTaq 16S/18S**
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 20µl, 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 culture (e.g. from *Saccharomyces cerevisiae*). Alternatively use 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:

Initial Denaturation: 95°C for 1min,

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

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):

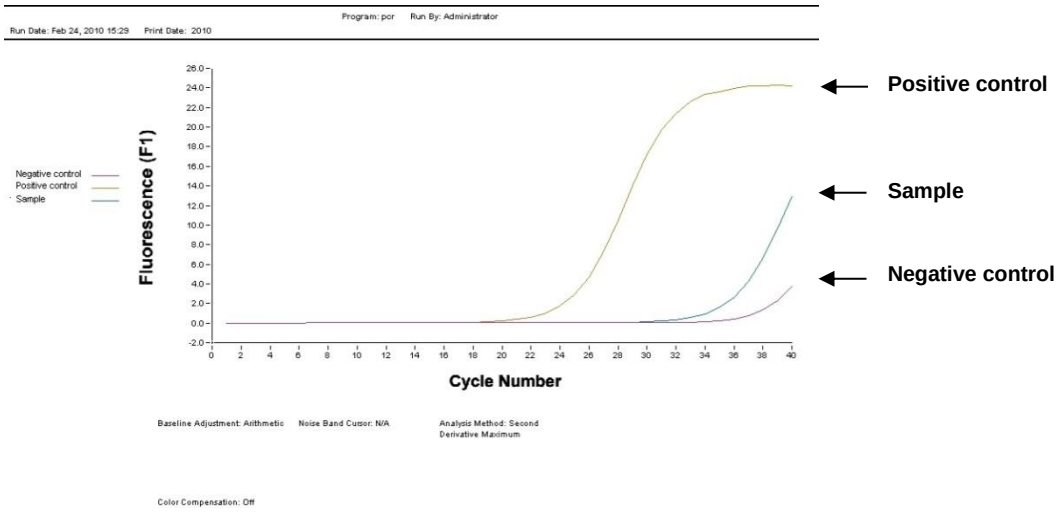

Fig. 3: Fluorescence curve: Please note that also the negative control may produce a signal due to primer dimer formation. It is always necessary to run a melting curve analysis (see Fig. 4) in order to distinguish between primer dimers and the specific amplicon.

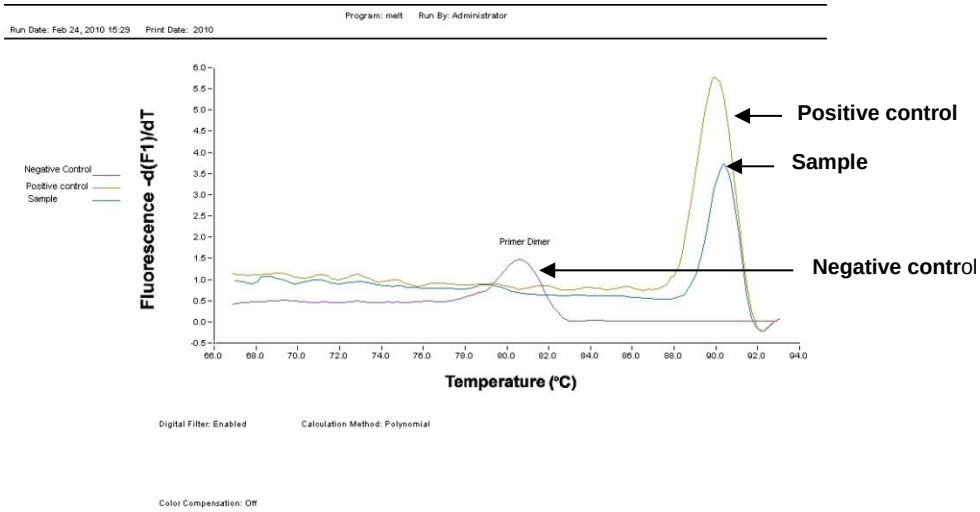

Fig. 4: Melting curve analysis from the Real time run above (Fig. 3). Note that the primer dimers in the negative control show a peak around 80°C, whereas a specific amplicon shows a peak around 87 to 91°C (see Pos. control and sample).

Identification

Follow the instructions given on page 4 (Identification of yeasts and other fungi 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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