# Onar® Bacteria

Bacteria Detection Kit for endpoint PCR

**INSTRUCTIONS FOR USE**

**FOR USE IN RESEARCH AND QUALITY CONTROL**

## Symbols


**Lot No.**


**Cat. No.**

**Expiry date**

**Storage temperature**

**Number of reactions**

**Manufacturer**

## INDICATION

The Onar® Bacteria Detection Kit is designed for direct detection of bacterial contamination in cell cultures, cell culture-derived biologicals, cell culture media and reagents. More generally, the Onar® Bacteria Detection Kit can be a valuable detection tool for all those applications, in which the presence of contaminant bacteria needs to be ascertained or excluded (e.g. water, lab reagents).

## TEST PRINCIPLE

Onar® Bacteria is based on conventional (or endpoint) polymerase chain reaction (PCR). By amplifying bacterial DNA, the PCR allows rapid and sensitive detection of bacterial contamination in various types of biological samples.

The kit contains lyophilized components such as the Bacteria Mix, the Positive Control DNA as well as Rehydration Buffer and the PCR Grade Water.

The Bacteria Mix contains a primer set targeting a highly conserved fragment of the 16S rRNA region of bacterial genomes. Among others, the following types of bacteria will be detected: *Pseudomonas, Actinomyces, Escherichia, Serratia, Porphyromonas, Fusobacteria, Staphylococcus, Streptococcus, Lactobacillus, Micrococcus, Bacillus, Klebsiella, Salmonella, Enterococcus, Mycobacterium, Legionella, Prevotella, Peptostreptococcus.* The PCR products will have a size of approx. 467 bp, depending on the detected bacterial species (*Micrococcus luteus* is 447 bp). The bands corresponding to the obtained amplicons will be easily visualized on an agarose gel with a conventional transilluminator (e.g. UV light).

The PCR mix also includes hot-start Taq polymerase and the Internal Control DNA. The Internal Control DNA gives rise to a 140 bp amplicon. The Internal Control DNA as well as the Positive Control DNA are tools to assess the assay performance. The kit contains dUTP instead of dTTP to facilitate the degradation of amplicon carry-over by use of uracil-DNA glycosylase (UNG). Thus, the probability of false positive results is minimized. Please note that UNG is not included in the Onar® Bacteria Kit.

## CONTENT

Each kit contains reagents for 25, 100, or 250 reactions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at +2 to +8 °C until use. The rehydrated components must be stored at ≤ -18 °C.

| Component | Quantity | | | Cap colour |
|---|---|---|---|---|
| | 25 reactions Cat.No. 12-1025 | 100 reactions Cat. No. 12-1100 | 250 reactions Cat. No. 12-1250 | |
| Bacteria Mix | 1 × vial lyophilized | 4 × vials lyophilized | 10 × vials lyophilized | red |
| Rehydration Buffer | 1 × vial 1.3 ml | 2 × vials 1.3 ml each | 5 × vials 1.3 ml each | blue |
| Positive Control DNA | 1 × vial lyophilized | 1 × vial lyophilized | 1 × vial lyophilized | green |
| PCR Grade Water | 1 × vial 2.0 ml | 1 × vial 2.0 ml | 1 × vial 2.0 ml | white |

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com).

## USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The Onar® Bacteria Detection Kit contains all necessary reagents to set-up the PCR test. Additional consumables and equipment are supplied by the user:

- PCR cycler
- Suitable nucleic acid-free PCR reaction tubes and 1.5 ml reaction tubes
- Microcentrifuge for 1.5 ml and PCR reaction tubes
- Pipettes with corresponding filter tips

## SPECIMEN

Please note that if your sample contains antibiotics, these may maintain the bacterial contamination at a level below the limit of detection of the test (below $10^3$ bacteria/ml). Therefore, prior to testing, the cells should be pre-cultured in the absence of antibiotics for at least one passage.

Samples should be heat-inactivated (at 95 °C for 10 min) prior to the test. Heat-inactivated samples may be stored at +2 to +8 °C for up to one week. Long-term storage must be at ≤ -18 °C. Repeated freezing and thawing should be avoided.

To avoid false positive results, we recommend using ultra-pure, DNA-free water, aerosol-resistant filter tips, and following the principles of good laboratory practice (e.g. wearing gloves at all time, see „Precautions“).

## PRECAUTIONS

The Onar® Bacteria Kit is for research use, only. The kit should be used by trained laboratory staff, only. All samples should be considered as potentially infectious and handled with all due care and attention. Always wear suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

Cross-contamination may lead to false positive results. The test should be performed according to good laboratory practice.

- Always use gloves
- Use face protection or avoid talking during setup and preparation of the samples
- Use unopened filter-tips boxes
- Do not use autoclaved PCR consumables such as tips and tubes
- Clean pipettes and surfaces regularly with DNA-removing agents (e.g. PCR Clean™)
- Use clean personal protective equipment like protective lab clothing
- Do not work at work benches with poorly maintained filters and avoid drafts (e.g. coworkers moving nearby) during pipetting

## ADDITIONAL NOTES

⇒ These instructions must be understood to successfully use the Onar® Bacteria Kit. The reagents supplied should not be mixed with reagents from different batches and used as an integral unit. The reagents of the kit must not be used beyond their expiry date.

⇒ Follow the exact protocol. Any deviation may affect the test method and can affect the results.

⇒ It is important to include control samples on a regular basis to monitor the reliability of your results. Positive and negative controls are essential in case of troubleshooting.

⇒ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA level (e.g. 3× $LOD_{95}$).

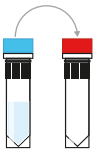
## PROCEDURE – OVERVIEW

### 1. Reagent Preparation

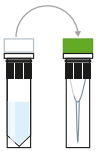
Bacteria Mix and Positive Control

520 µl

300 µl



Bacteria Mix



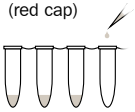
Positive Control

for 5 min RT
briefly
for 5 sec

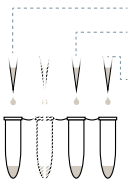
### 2. Preparation of PCR Reactions

loading the test tubes

aliquot 20 µl Bacteria Mix (red cap)



+ 5 µl sample
+ 5 µl Positive Control (green cap)
+ 5 µl PCR Grade Water (white cap) (Negative Control)



briefly

### 3. Start PCR Reaction

| | |
|---|---|
| 1 cycle | 94 °C for 2 min |
| 35 cycles | 94 °C for 30 sec |
| | 55 °C for 30 sec |
| | 72 °C for 30 sec |
| hold | +4 to +8 °C |

100 bp DNA Ladder
Negative Control
Positive Control
Inhibited Sample
Negative Sample
Positive Sample

Rehydration Buffer
Bacteria Mix
PCR Grade Water
Positive Control

incubate
vortex
centrifuge
add

This procedure overview is not a substitute for the detailed manual.

## PROCEDURE - STEP BY STEP

The test should be carried out with negative and positive controls and samples in duplicate. All reagents and samples must be equilibrated to +2 to +8 °C prior to use. After reconstitution, the reagents must be stored at ≤ -18 °C. Repeated freezing and thawing should be avoided and re-constituted controls (control and positive control) stored in aliquots.

### 1. Reagent preparation

| | | | |
|---|---|---|---|
| 1. | Bacteria Mix<br>Positive Control DNA | red cap<br>green cap | Spin down all lyophilized components at max speed for 5 sec. |
| 2. | Bacteria Mix | red cap | Add 520 µl Rehydration Buffer (blue cap)<br>For sample kit (10 reactions) only: add 208 µl Rehydration Buffer |
| 3. | Positive Control DNA | green cap | Add 300 µl PCR Grade Water (white cap) |
| 4. | Bacteria Mix<br>Positive Control DNA | red cap<br>green cap | Incubate 5 min at room temperature |
| 5. | Bacteria Mix<br>Positive Control DNA | red cap<br>green cap | Vortex briefly and spin for 5 sec |

### 2. Reaction mix preparation

| | | |
|---|---|---|
| 1. | Aliquot 20 µl of the Bacteria Mix to each PCR tube | |
| 2. | Negative Controls: | add 5 µl PCR Grade Water (white cap) |
| 3. | Samples: | add 5 µl of heat-inactivated sample |
| 4. | Positive Control: | add 5 µl Positive Control DNA (green cap) |
| 5. | Close the tubes tightly and spin briefly. Proceed with the PCR. | |

### 3. Start PCR amplification

| | | |
|---|---|---|
| 1. | Place PCR tubes in the PCR cycler and close the lid. | |
| 2. | Program the PCR cycler: | |
| | 1 cycle | 94 °C for 2 min |
| | 35 cycles | 94 °C for 30 sec<br>55 °C for 30 sec<br>72 °C for 30 sec |
| | Hold between | +4 °C and +8 °C |
| 3. | Start the program. | |

## 4. Agarose gel electrophoresis

| | | | |
|---|---|---|---|
| 1. | Cast a 1.5 % or 2 % agarose gel including a suitable DNA stain (maximal 5 mm thick, 5 mm comb). | | |
| 2. | Load 5 µl of each PCR reaction, mixed with bromophenol blue loading buffer per lane (only bromophenol blue in low concentrations should be used). | | |
| 3. | Stop electrophoresis after 2 cm (or more) run distance (depending on the used electrophoresis chamber, run for approx. 20 minutes at 100 V). | | |
| 4. | Analysis: | Internal control | 140 bp |
| | | Bacteria | ~467 bp |

A characteristic gel image is shown below. Representative contaminated samples with the listed species are represented.

Negative sample

Inhibited sample

Contaminated sample with: Salmonella enterica

Contaminated sample with: Escherichia coli

Contaminated sample with: Pseudomonas aeruginosa

Contaminated sample with: Legionella pneumophila

Contaminated sample with: Bacillus subtilis

Positive control

Negative control

Marker

**NOTE:** A weak indistinct smear is not considered as positive amplification and may result from non-bacterial DNA background. The intensity of the internal control band will fade out with increasing amounts of bacterial amplicons, as for example in case of strong sample contaminations.

### Cross-reactivity

Cross-reactivity with DNA of eukaryotic origin could not be found. The PCR assay will not detect any yeast, fungi or viruses.

## DATA INTERPRETATION

The Internal Control DNA will result in the appearance of a distinct 140 bp band in every lane, indicating a successfully performed PCR. Due to competition between the internal control and the target reaction, the internal control band will fade out when large amounts of primary target (bacterial amplicons) are initially present. Consequently, the internal control band can be absent in the positive control reaction, due to the high concentration of positive control DNA.

Rarely, unspecific PCR products can form and become visible on the gel as faint, diffuse bands of different sizes that do not indicate positive results. These unspecific amplifications are mainly caused by unspecific annealing reactions, which can depend on the sample-specific DNA load and lead to the formation of primer-dimers or PCR artifacts.

These unexpected results would in any case be easily recognizable as their unspecific products would be visualized in a different size range than the specific product.

The following matrix will help to interpret the PCR result:

| **Detection of bacteria amplicon at ~467 bp** | **Internal control amplicon at 140 bp** | **Interpretation** |
|---|---|---|
| positive | irrelevant | Bacteria present in the sample |
| negative | negative | PCR inhibition |
| negative | positive | No bacteria detectable in the sample |

## APPENDIX

*Limited Product Warranty*

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from the use, the results of use, or the inability to use this product.

*Trademarks*

Onar, Venor, Mynox, and ZellShield are registered trademarks and PCR Clean, Mycoplasma Off, and WaterShield are trademarks of Minerva Biolabs GmbH.

## Related Products

**MB Taq DNA Polymerase**

| | | |
|---|---|---|
| 53-0050/-0100/-0200/-0250 | MB Taq DNA Polymerase (5 U/µl) | 50/100/200/250 units |
| 53-1050/-1100/-1200/-1250 | MB Taq DNA Polymerase (1 U/µl) | 50/100/200/250 units |

**Contamination Control Kits for conventional PCR**

| | | |
|---|---|---|
| 11-1025/-1050/-1100/-1250 | Venor®GeM Classic Mycoplasma Detection Kit | 25/50/100/250 reactions |
| 11-7024/-7048/-7096/-7240 | Venor®GeM Advance Mycoplasma Detection Kit | 24/48/96/240 reactions |
| 11-8025/-8050/-8100/-8250 | Venor®GeM OneStep Mycoplasma Detection Kit | 25/50/100/250 reactions |

**Sample Preparation**

| | | |
|---|---|---|
| 56-1010/-1050/-1200 | Venor®GeM Sample Preparation Kit | 10/50/200 extractions |

**Mycoplasma Elimination**

| | | |
|---|---|---|
| 10-0200/-0500/-1000 | Mynox® Mycoplasma Elimination Reagent | 2/5/10 treatments |
| 10-0201/-0501/-1001 | Mynox® Gold Mycoplasma Elimination Reagent | 2/5/10 treatments |

**PCR Quantification Standards, 1 × $10^8$ genomes / vial**

| | |
|---|---|
| 52-0112 | *Mycoplasma orale* |
| 52-0115 | *Mycoplasma gallisepticum* |
| 52-0116 | *Acholeplasma laidlawii* |
| 52-0117 | *Mycoplasma fermentans* |
| 52-0119 | *Mycoplasma pneumoniae* |
| 52-0124 | *Mycoplasma synoviae* |
| 52-0129 | *Mycoplasma arginini* |
| 52-0130 | *Mycoplasma hyorhinis* |
| 52-0164 | *Spiroplasma citri* |

See MB homepage for further available species

**Genomic DNA Extracts - Specificity Standards, 10 ng ± 2 ng / vial**

| | |
|---|---|
| 51-0129 | *Mycoplasma arginini* |
| 51-0162 | *Mycoplasma arthritidis* |
| 51-0117 | *Mycoplasma fermentans* |
| 51-0115 | *Mycoplasma gallisepticum* |
| 51-0195 | *Mycoplasma genitalium* |
| 51-0111 | *Mycoplasma hominis* |
| 51-0130 | *Mycoplasma hyorhinis* |
| 51-0112 | *Mycoplasma orale* |
| 51-1746 | *Mycoplasma penetrans* |
| 51-0119 | *Mycoplasma pneumoniae* |
| 51-0113 | *Mycoplasma salivarium* |
| 51-0124 | *Mycoplasma synoviae* |
| 51-0164 | *Spiroplasma citri* |
| 51-0231 | *Staphylococcus aureus* |

See MB homepage for further available species

**PCR Clean™**

| | | |
|---|---|---|
| 15-2025/-2200/-2500 | DNA Decontamination Reagent, spray bottle/refill bottles/canister | 250 ml/4×500 ml/5 l |
| 15-2001/-2002 | Wipes in dispenser box / refill packs | 50 wipes /5×50 wipes |

**Mycoplasma Off™**

| | | |
|---|---|---|
| 15-1000/-5000 | Surface Disinfectant Spray, spray bottle | 1 l/5 l |
| 15-1001/-5001 | Surface disinfectant Wipes in dispenser box / refill packs | 50 wipes /5×50 wipes |

**ZellShield®**

| | | |
|---|---|---|
| 13-0050/-0150 | Contamination Prevention Reagent 100 × concentrate | 50 ml/3×50 ml |

**WaterShield™**

| | | |
|---|---|---|
| 15-3015/-3020/-3050 | Water Disinfection Additive for incubators and water baths, 200 × concentrate | 15×10 ml/3×50 ml/ 500 ml |

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