

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, 50, 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	50 reactions Cat. No. 12-1050	100 reactions Cat. No. 12-1100	250 reactions Cat. No. 12-1250	
Bacteria Mix	1 × vial lyophilized	2 × vials lyophilized	4 × vials lyophilized	10 × vials lyophilized	red
Rehydration Buffer	1 × vial 1.3 ml	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	1 × vial lyophilized	green
PCR Grade Water	1 × vial 2.0 ml	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 10³ 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

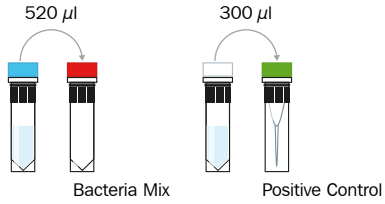
ADDITIONAL NOTES

- ⇒ 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. $3 \times \text{LOD}_{95}$).

PROCEDURE – OVERVIEW

1. Reagent Preparation

⊕ Bacteria Mix and Positive Control

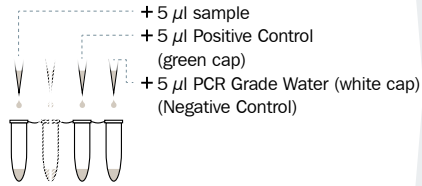
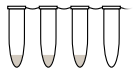


⌚ for 5 min RT
🌀 briefly
⊕ for 5 sec

2. Preparation of PCR Reactions

loading the test tubes

aliquot 20 µl Bacteria Mix (red cap)



⊕ 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

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 reconstituted controls (control and positive control) stored in aliquots.

1. Reagent preparation

1.	Bacteria Mix Positive Control DNA	red cap 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) 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 Positive Control DNA	red cap green cap	Incubate 5 min at room temperature
5.	Bacteria Mix Positive Control DNA	red cap 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.	

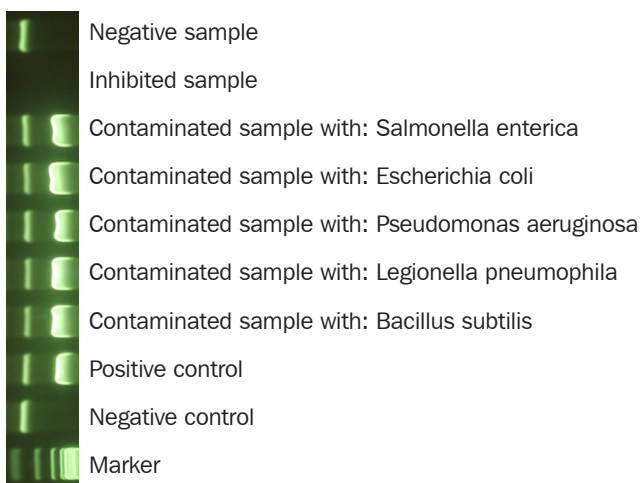
3. Start PCR amplification

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 55 °C for 30 sec 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 μ l 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.



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/ μ l)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB Taq DNA Polymerase (1 U/ μ l)	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
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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 × 10⁸ genomes / vial

52-0112	<i>Mycoplasma orale</i>
52-0115	<i>Mycoplasma gallisepticum</i>
52-0116	<i>Acholeplasma laidlawii</i>
52-0117	<i>Mycoplasma fermentans</i>
52-0119	<i>Mycoplasma pneumoniae</i>
52-0124	<i>Mycoplasma synoviae</i>
52-0129	<i>Mycoplasma arginini</i>
52-0130	<i>Mycoplasma hyorhinis</i>
52-0164	<i>Spiroplasma citri</i>

See MB homepage for further available species

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

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

See MB homepage for further available species

PCR Clean™

15-2025/-2200/-2500	DNA Decontamination Reagent, spray bottle/refill bottles/canister	250 ml/4x500 ml/5 l
15-2001/-2002	Wipes in dispenser box / refill packs	50 wipes /5x50 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 /5x50 wipes

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100 × concentrate	50 ml/3x50 ml
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WaterShield™

15-3015/-3020/-3050	Water Disinfection Additive for incubators and water baths, 200 × concentrate	15x10 ml/3x50 ml/ 500 ml
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