



iQ-Check® Free DNA Removal Solution for *Legionella*

- Easy to use
- Improves confidence in results
- Safe reagent
- Suitable with Aquadien short protocol

One of the primary challenges when performing PCR for environmental water testing is the potential presence of free DNA, which can lead to an overestimation of the level of targeted DNA sequences. When quantifying *Legionella*, culture and PCR levels can differ from each other by up to 2 logs.

To improve *Legionella* testing in water, a supplemental step using the iQ-Check Free DNA Removal Solution (FDRS) can be introduced into the Aquadien™ DNA Extraction and Purification Kit. FDRS (catalog #3594970) is easily integrated in the Aquadien DNA Extraction Kit workflow and provides an ideal way to remove free DNA from water samples prior to PCR analysis.

While the DNA in intact and living cells is protected, FDRS will degrade the free DNA from dead and lysed cells in the sample.

Principle

The degradation of free DNA is performed by a selected reagent and its specific activation buffer under optimized conditions (Figure 1). Then the Aquadien Extraction Kit R1 solution associated with thermal lysis inactivates the enzyme, allowing for the extraction of DNA from intact and living cells.

Using the Aquadien Free DNA Removal Solution protocol results in up to a 2.5 log reduction in signal from free DNA.

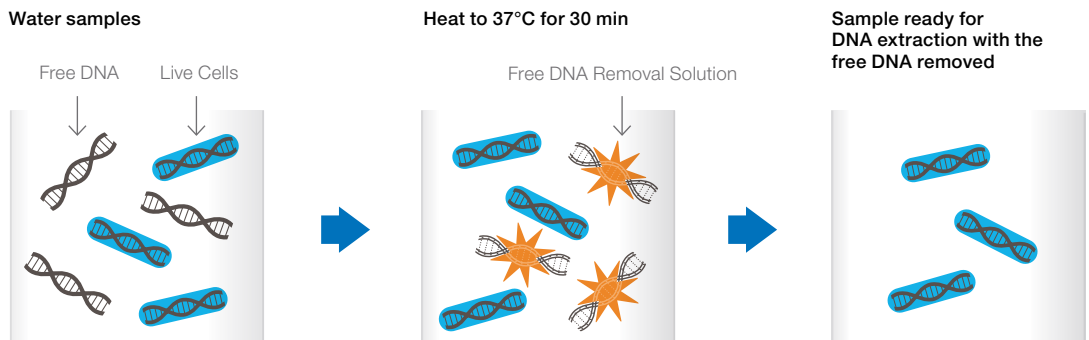
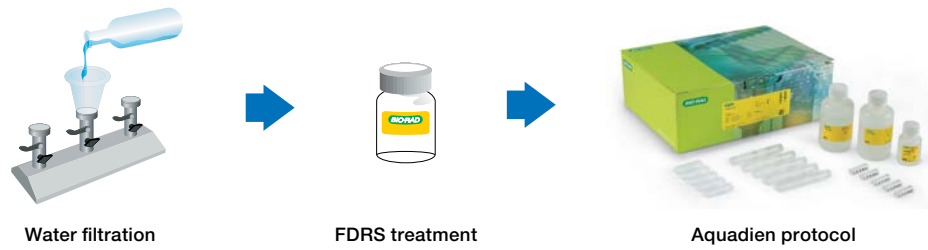


Fig. 1. Treatment with the iQ-Check Free DNA Removal Solution. Free DNA from dead and lysed bacteria is present with live bacteria in the water sample. The iQ-Check FDRS removes the free DNA from the water sample, leaving viable bacteria to be detected by PCR. The FDRS is then inactivated during the DNA extraction step in the Aquadien Kit workflow.

Protocol

Filter a volume of water sample. Add 40 µl of activated FDRS to 460 µl of *Legionella* DNA Free Water. Incubate 30 min at 37°C. Proceed with the Aquadien protocol.



Performance

Water samples spiked with free DNA and live bacteria simulate different amounts of free genomes/filter. Treatment with the FDRS reduces the signal of free DNA/lysed dead cells up to 2.5 log without affecting live cells (Table 1).

Table 1. Evaluation of FDRS action on spiked free gDNA and live bacteria on *Legionella* spp.

	Genome/Filter without FDRS	Genome/Filter with FDRS	Δ log without FDRS vs. with FDRS	Interpretation
Spiked gDNA*	1.5 x 10 ⁶	7.3 x 10 ³	-2.3 ± 0.4	FDRS action
Live bacteria**	2.0 x 10 ⁵	1.0 x 10 ⁵	-0.20	No FDRS action

* Intermediate precision of FDRS action on the protocol; 3 days of testing; mean of three filters/day.

** Mean of two filters by concentration.

An evaluation of the iQ-Check Free DNA Removal Solution was performed on water from six clean and clogged environmental samples using the iQ-Check Quanti Real-Time PCR Quantification Kit for *Legionella* spp. or *L. pneumophila*. For five of these samples, free DNA was removed with FDRS treatment. Two samples achieved equivalency between PCR and culture methods. Differences between PCR and culture results could be due to viable non-culturable (VBNC) bacteria that were not detectable in culture. Data representing the differences between sample results are compared in Table 2.

Table 2. FDRS action on environmental water samples and comparison between qPCR and culture results on *Legionella* spp. and *L. pneumophila* targets.

Targets	qPCR without FDRS, log GU/L	qPCR with FDRS, log GU/L	FDRS Action, log	Culture, log CFU/L	Δ log Culture vs. qPCR with FDRS	FDRS Action and Observation	Interpretation
<i>Legionella</i> spp.	7.5	6.5	-1.0	4.7	-1.8	Free DNA removed; high presence of VBNC	VBNC not detected by culture
<i>Legionella</i> spp.	7.1	5.6	-1.5	4.7	-0.9	Free DNA removed; medium presence of VBNC	VBNC not detected by culture
<i>Legionella</i> spp.	7.6	5.2	-2.4	4.7	-0.5	Free DNA removed; low presence of VBNC	VBNC not detected by culture
<i>L. pneumophila</i>	6.1	5.4	-0.7	5.5	0.1	Free DNA removed	PCR and culture are equivalent
<i>L. pneumophila</i>	4.4	3.7	-0.7	3.9	0.2	Free DNA removed	PCR and culture are equivalent
<i>L. pneumophila</i>	5.2	5.0	-0.2	3.7	-1.4	No free DNA; no presence of VBNC	VBNC not detected by culture

Conclusion

Adding the FDRS step to the Aquadien DNA Extraction and Purification Kit removes free DNA that is present in water samples, leading to equivalency between PCR and culture results when VBNC are not present.

Aquadien FDRS Protocol

The Aquadien Free DNA Removal Solution is part of the Aquadien Short DNA Extraction Protocol.

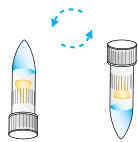


- Filter water sample

460 μ l water + 40 μ l FDRS



- Add the filter in a tube containing 460 μ l *Legionella* DNA Free Water and 40 μ l FDRS



37°C



- Invert the tube several times to homogenize (do not vortex)
- Incubate at 37°C for 30 min

Add 500 μ l R1



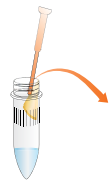
- Add 500 μ l R1 to inactivate FDRS for DNA extraction
- Vortex for 10 sec



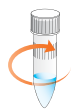
95°C



- Incubate for 15 min at 95°C and 1,300 rpm in a shaking heating block

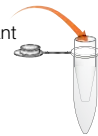


900 g



- Take the filter out carefully and press it on the walls of the tube to recover all the solution
- Centrifuge at 900 g for 3 min
The DNA is contained in the 800 μ l of supernatant

Add 500 μ l supernatant



6,000 g



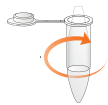
- Place the purification column in a collector vial
- Apply 500 μ l of the supernatant on the purification column
- Centrifuge at 6,000 g for 10 min

100 μ l R2

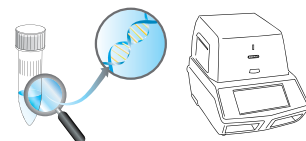


New collection vial

1,000 g



- Add 100 μ l of R2 solution, discard the collection vial
- Cover the purification column with a clean collector vial and invert the two together
- Centrifuge at 1,000 g for 3 min
- Discard the purification column



- 100 μ l of purified DNA should be obtained
- Use 5 μ l of the extracted DNA for real-time PCR analysis

Ordering Information

Catalog #	Description
3594970	iQ-Check Free DNA Removal Solution , iQ-Check Free DNA Removal Reagent, 1 vial (175 mg lyophilized powder), iQ-Check 10x Activation Buffer, 1 tube (9 ml)
12006823	Legionella DNA Free Water , 1 L bottle

Associated Kits and Reagents

3578121	Aquadien DNA Extraction and Purification Kit , 96 reactions
3578102	iQ-Check Quanti Legionella spp. Real-Time PCR Quantification Kit , 96 reactions
3578103	iQ-Check Quanti L. pneumophila Real-Time PCR Quantification Kit , for up to 96 samples
3578104	iQ-Check Screen Legionella spp. Real-Time PCR Detection Kit , 96 reactions
3578105	iQ-Check Screen L. pneumophila Real-Time PCR Detection Kit , 96 reactions

Instruments

3600037	CFX96 Touch™ Deep Well Real-Time PCR Detection System , 96 wells
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Bio-Rad's thermal cyclers and real-time thermal cyclers are covered by one or more of the following U.S. patents or their foreign counterparts owned by Eppendorf AG: U.S. Patent Numbers 6,767,512 and 7,074,367.

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