



iQ-Check® Free DNA Removal Solution

- Increased confidence in results
- Seamless integration with iQ-Check Real-Time PCR Detection Kits
- Easy to use

Since PCR became the method of choice for routine testing, one of its major challenges has been the detection of dead cells. With the iQ-Check methods, 10^4 to 10^5 dead cells/25 g of matrix would be needed to get a PCR positive result. The Free DNA Removal Solution, which can easily be integrated with iQ-Check DNA extraction workflows, provides an ideal way to remove free DNA from food and environmental samples prior to PCR analysis.

While the DNA in intact and living cells is protected, the free DNA from the sample, and any that is present due to different industrial

processes, will be degraded. This enzymatic solution performs as well as ethidium monoazide (EMA) or propidium monoazide (PMA) treatments but in a much easier and simpler way.

Principle

The degradation of free DNA is performed by a selected enzyme and its specific activation buffer under optimized conditions. This enzymatic treatment ensures that the enriched broths are devoid of free DNA prior to DNA extraction (Figure 1). Then the iQ-Check Lysis Buffer associated with thermal lysis inactivates the enzyme, allowing for the extraction of DNA from intact and living cells.

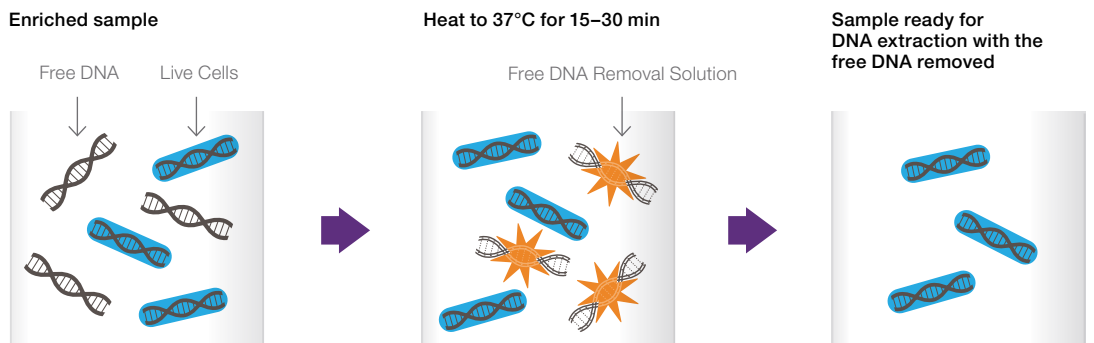


Fig. 1. Treatment with the Free DNA Removal Solution. Free DNA from dead bacteria or phage-based intervention is present with live bacteria in the enriched sample. The iQ-Check Free DNA Removal Solution removes the free DNA from the enriched sample leaving viable bacteria to be detected by PCR. The Free DNA Removal Solution is inactivated during the DNA extraction step in the iQ-Check method.

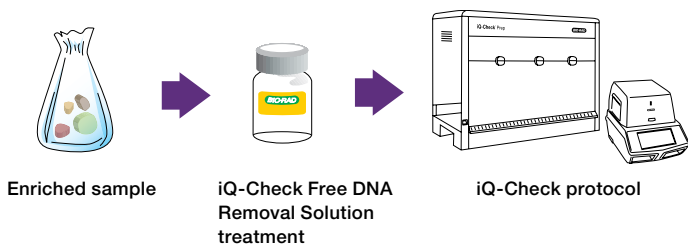
The iQ-Check Free DNA Removal Solution is recommended for analysis of:

- Heat processed/cooked matrices (for example, milk powders and subcomponents)
- Products treated with phage application (for example, Listex and Salmorex)
- Environmental samples subjected to cleaning agents and potentially carrying dead cells

Using the iQ-Check Free DNA Removal Solution results in an average 2–3 log (approximately 6 Cq values) reduction in signal from free DNA.

Protocol

Enrich the sample according to the appropriate iQ-Check method. Add 10 µl of activated iQ-Check Free DNA Removal Solution to 100 µl of enriched sample. Incubate 15–30 min at 37°C. Proceed with the iQ-Check method.



Performance

Treatment with the iQ-Check Free DNA Removal Solution reduces the signal of dead cells/free DNA by an average of 2 logs or 6 Cq values. Contamination with 4 genomes/PCR reaction corresponds to approximately 4×10^5 dead cells/25 g of matrix. With the Free DNA Removal Solution, the signal from this level of contamination is completely eliminated (Table 1).

Table 1. Evaluation of the signal detected from serial dilution of dead *Listeria* spp. cells tested with the iQ-Check *Listeria* spp. Real-Time PCR Detection Kit in the presence and absence of Free DNA Removal Solution treatment.

Dead cell contamination, genomes/PCR reaction	Signal without iQ-Check Free DNA Removal Solution, Cq values	Signal with iQ-Check Free DNA Removal Solution, Cq values
400	29.5	Not detected
40	31.7	Not detected
4	35.4	Not detected

An independent evaluation of the iQ-Check Free DNA Removal Solution was performed on four different matrices using the iQ-Check *Listeria* spp. Real-Time PCR method in accordance with current AOAC guidelines (Table 2). Using probability of detection (POD) analysis, no statistically significant differences were observed between the method using the Free DNA Removal Solution and the reference method for all the matrices tested. The iQ-Check Free DNA Removal Solution does not affect the detection of living cells.

This protocol has also been successfully tested on milk powders and subcomponents. Contact your Bio-Rad sales representative for more information.

Table 2. Independent evaluation of the Free DNA Removal Solution using the iQ-Check *Listeria* spp. Real-Time PCR method in accordance with current AOAC guidelines.

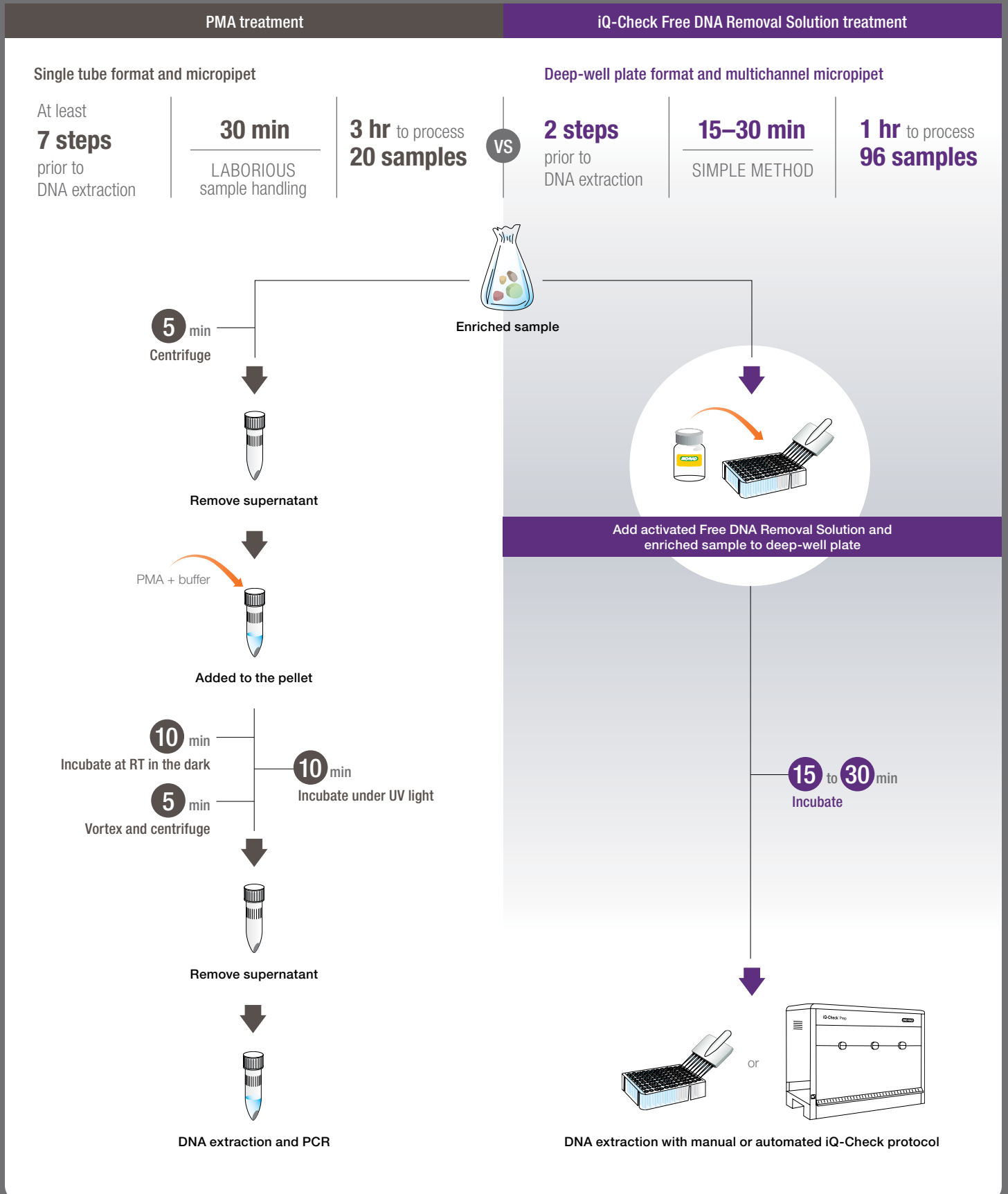
Matrix	Strain	MPN and CFU/ Test Portion	Number of Samples	iQ-Check <i>Listeria</i> spp. Kit		Reference Method Positive	Equivalency Criteria
				Presumptive Positive	Confirmed Positive		
Deli ham	<i>Listeria monocytogenes</i> ATCC 19115	0	5	0	0	0	Pass
		0.44	20	9	9	6	Pass
		3.01	5	5	5	5	Pass
Cheese	<i>Listeria welshimeri</i> ATCC 35897	0	5	0	0	0	Pass
		0.55	20	9	9	8	Pass
		3.01	5	5	5	5	Pass
Stainless steel	<i>Listeria innocua</i> ATCC 33091 and <i>Enterococcus faecalis</i> ATCC 29212	0	5	0	0	0	Pass
		38 and 420	20	5	5	6	Pass
		200 and 2,100	5	5	5	5	Pass
Sealed concrete	<i>Listeria monocytogenes</i> ATCC 7644	0	5	0	0	0	Pass
		54	20	7	7	7	Pass
		170	5	5	5	5	Pass

MPN, most probable number. CFU, colony forming units.



WORKFLOW COMPARISON

The following diagram compares the iQ-Check Free DNA Removal protocol to current PMA treatment methods. Both are equally efficient, but treatment with the iQ-Check Free DNA Removal Solution is easier and can be carried out in fewer steps.



Ordering Information

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

iQ-Check Real-Time PCR Kits

3578135	iQ-Check <i>Campylobacter</i> Kit, 96 reactions
3578137	iQ-Check <i>Cronobacter</i> Kit, 96 reactions
3578114	iQ-Check <i>E.coli</i> O157:H7 Kit, 96 reactions
3578124	iQ-Check <i>Listeria monocytogenes</i> II Kit, 96 reactions
3578113	iQ-Check <i>Listeria</i> spp. Kit, 96 reactions
3578123	iQ-Check <i>Salmonella</i> II Kit, 96 reactions
3578142	iQ-Check <i>S. Enteritidis</i> Kit, 96 reactions
3578139	iQ-Check STEC VirX Kit, 96 reactions
Coming soon	iQ-Check <i>S. Enterobacteriaceae</i> Kit
Coming soon	iQ-Check <i>S. Typhimurium</i> Kit

Instruments

3600037	Industrial Diagnostic CFX96 Touch™ Deep Well Real-Time PCR Detection System , 96 wells (3594990 in Europe)
3594911	iQ-Check Prep System

Catalog #	Description
Media for Sample Enrichment	
3554179	Buffered Peptone Water Broth , 225 ml x 6 bottles
3564684	Buffered Peptone Water Broth , 500 g
3555797	Fraser ½ Broth , 225 ml x 6 bottles
3553454	Trypto-Casein-Soy (TCS) Broth , 10 ml x 25 tubes
3555703	Listeria Special Broth (LSB) , 225 ml x 6 bottles
3564703	Listeria Special Broth (LSB) , 500 g
3564753	Listeria Special Broth (LSB) , 5 kg
3564001	STEC Enrichment Broth , 500 g

Chromogenic Media for Confirmation

3564748	RAPID <i>E.coli</i> O157:H7 Dehydrated Base , 100 g
3564744	RAPID <i>Listeria</i> spp. Dehydrated Base , 500 g
3563694	RAPID <i>L.mono</i> Agar , 90 mm x 20 plates
3563971	RAPID <i>Sakazakii</i> Agar , 90 mm x 20 plates
3563963	RAPID <i>Salmonella</i> Agar , 90 mm x 120 plates

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Bio-Rad's 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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