

iQ-Check® Free DNA Removal Solution for Food, Water, and Environmental Samples

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

For laboratory use only.

Introduction

Since PCR became the method of choice for routine testing, one of its major challenges has been the potential detection of dead cells. For iQ-Check methods, the Free DNA Removal Solution, which can easily be integrated with iQ-Check and Aquadien™ DNA extraction workflows, provides an ideal way to remove free DNA from food, water, 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.

iQ-Check Free DNA Removal Solution for Enriched Food and Environmental Samples

Principle

The degradation of free DNA is performed by a selected enzyme and its specific buffer under optimized conditions. This enzymatic treatment ensures that the enriched broths are devoid of free DNA prior to DNA extraction. Then the iQ-Check Lysis Buffer associated with thermal lysis inactivates the enzyme, allowing for the extraction of DNA from intact and living cells. This enzymatic solution performs as well as ethidium monoazide (EMA) or propidium monoazide (PMA) treatments but in a much easier and simpler way.

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 interventions
- Environmental samples submitted 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 quantification cycle [Cq] values) reduction in signal from free DNA. A complete performance report is available on request.

Kit Components

The iQ-Check Free DNA Removal Solution Kit contains sufficient reagents for 10 x 96 tests.

Reference	ID Reagent	Quantity Provided
G1	iQ-Check Free DNA Removal Reagent	1 vial, 175 mg lyophilized powder
G2	iQ-Check 10x Activation Buffer	1 vial, 9 ml

Shelf Life and Storage

Once received, the kit must be stored at 2–8°C. An unopened kit can be used until the expiration date indicated on the labels.

The shelf life of activated solution (rehydrated G1 + G2) is 24 hr at room temperature, 7 days at 2–8°C, and 3 months at –20°C. Before reusing, allow the vial to thaw to room temperature.

It is recommended to aliquot the activated reagent: 1 ml is needed for 96 tests when performing the easy DNA extraction protocol (10 µl reagent/100 µl of enriched sample).

Required Materials Not Supplied

This is a nonexhaustive list.

Equipment

- 1,000 µl micropipet
- Thermoshaker capable of maintaining 37 ± 2°C, Bio-Rad catalog #3594986

Supplies

- Sterile distilled water or Milli-Q water
- Sterile filter tips, adaptable to 1,000 µl

- Specific for extraction in a deep well plate
 - 1 ml deep well microplate, Bio-Rad catalog #3594900
 - X-Pierce Film, Bio-Rad catalog #3593977 (#3600040 in U.S. and Canada)
- Powder-free gloves
- Decontaminating agent, such as DNA AWAY or RNase AWAY
- Visit bio-rad.com/iqcheck for iQ-Check food kits

Precautions

Precautions and recommendations for best results.

- This protocol must be performed by adequately trained personnel
- All potentially infectious material should be autoclaved before disposal
- The quality of results depends on strict compliance with the following Good Laboratory Practice, especially concerning PCR:
 - The laboratory equipment (pipets, tubes, etc.) must not circulate from one workstation to another
 - It is essential to use a positive control and a negative control for each series of amplification reactions
 - Do not use reagents after their expiration date
 - Periodically verify the accuracy and precision of pipets and the correct functioning of the instruments
 - Change gloves often, especially if you suspect they are contaminated
 - Clean work spaces periodically with at least 5% bleach and a decontaminating agent like DNA AWAY
 - Use powder-free gloves
- It is strongly advised to follow the general requirements described in the standard EN ISO 22174:2005, “Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions”

See the safety data sheet at foodscience.bio-rad.com for product safety information.

Validations and Tested Applications

Scope (method and matrices)	Enrichment	DNA Extraction		Certification
		Method	Format	
iQ-Check <i>Listeria</i> spp.	LSB	Easy	Tube; deep well microplate	AOAC-RI
Stainless steel, sealed concrete, sliced deli ham, natural cheese, 125 g	25 ± 1 hr at 30°C			

This protocol has been successfully tested on milk powders (and subcomponents), cheese, and environmental samples. Contact your Bio-Rad sales representative for more information.

Protocol

Please read the entire protocol before starting the assay.

A. Sample Enrichment

Follow the recommended instructions indicated in each iQ-Check Kit insert.

B. iQ-Check Free DNA Removal Treatment

Activation of the iQ-Check Free DNA Removal Solution

Reagents G1 and G2 can be used until the expiration date indicated on the vials.

- Rehydrate the freeze-dried reagent G1 in 1 ml of distilled or Milli-Q water for 5 to 10 min at room temperature. Gently mix by inverting the vial. **DO NOT VORTEX**
- After hydration, transfer G1 (1 ml) to the activation buffer vial (G2, 9 ml). Gently mix by inverting the vial

The solution is now activated and ready to use. You will need 1 ml of the activated solution (G1 + G2) to perform 96 tests using the Easy DNA Extraction Protocol (10 µl reagent/100 µl enriched sample).

To guarantee stability, pipet 10 µl of activated reagent into the bottom of each well of a 96-deep well microplate (Bio-Rad catalog #3594900) for immediate use or aliquot into 1 ml tubes for storage.

Treatment with the Activated Solution (G1 + G2)

Before starting the test, turn on the thermoshaker and set it to 37 ± 2°C.

- Use the plate prepared in the step above or dispense 10 µl of activated solution (G1 + G2) into as many wells of an empty deep well microplate as there are samples to be analyzed
- Add 100 µl of decanted enriched sample per well. Avoid matrix debris or fat layers
- Seal the deep well microplate with the X-Pierce Sealing Film
- Incubate the sealed deep well microplate in the thermoshaker **WITHOUT** shaking at 37 ± 2°C for 15 to 30 min

C. iQ-Check Protocols

Proceed to the Easy DNA Extraction Protocols (I or II) using the 100 µl of treated enriched sample in 100 µl of lysis reagent following Bio-Rad's recommendations in the user guide of the iQ-Check PCR Kit.

Be sure that the thermoshaker has reached 95–100°C to properly inactivate the iQ-Check Free DNA Removal Reagent.

iQ-Check Free DNA Removal Solution for *Legionella*

Principle

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

The iQ-Check Free DNA Removal Solution is recommended for analysis of environmental samples for *Legionella* testing. Using the iQ-Check Free DNA Removal Solution results in up to 2.5 log (approximately 6 Cq values) reduction in signal from free DNA.

Kit Components

The iQ-Check Free DNA Removal Solution Kit contains sufficient reagents for 250 tests for the Aquadien Free DNA Removal Solution (FDRS) protocol.

Reference	ID Reagent	Quantity Provided
G1	iQ-Check Free DNA Removal Reagent	1 vial, 175 mg lyophilized powder
G2	iQ-Check 10x Activation Buffer	1 vial, 9 ml

Shelf Life and Storage

Once received, the kit must be stored at 2–8°C. An unopened kit can be used until the expiration date indicated on the labels.

The shelf life of activated solution (rehydrated G1 + G2) is 24 hr at room temperature, 7 days at 2–8°C, and 3 months at –20°C. Before reusing, allow the vial to thaw to room temperature.

It is recommended to aliquot the activated reagent for storage (40 µl are necessary for each sample).

Required Materials Not Supplied

This is a nonexhaustive list.

Equipment

- 1,000 µl micropipet
- Thermoshaker capable of maintaining 37 ± 2°C, Bio-Rad catalog #3594986

Supplies

- Legionella* DNA-Free Water, Bio-Rad catalog #12006823
- Sterile filter tips, adaptable to 1,000 µl
- 2 ml tubes for Aquadien short protocol
- Powder-free gloves
- Decontaminating agent, such as DNA AWAY or RNase AWAY

Precautions

Precautions and recommendations for best results.

- This protocol must be performed by adequately trained personnel
- All potentially infectious material should be autoclaved before disposal
- The quality of results depends on strict compliance with the following Good Laboratory Practice, especially concerning PCR
 - The laboratory equipment (pipets, tubes, etc.) must not circulate from one workstation to another
 - It is essential to use a positive control and a negative control for each series of amplification reactions
 - Do not use reagents after their expiration date
 - Periodically verify the accuracy and precision of pipets and the correct functioning of the instruments
 - Change gloves often, especially if you suspect they are contaminated
 - Clean work spaces periodically with at least 5% bleach and a decontaminating agent like DNA AWAY
 - Use powder-free gloves

See the safety data sheet at foodscience.bio-rad.com for product safety information.

Protocol

Please read the entire protocol before starting the assay.

Follow the recommended instructions indicated in each iQ-Check Kit insert.

A. iQ-Check Free DNA Removal Treatment

Activation of the iQ-Check Free DNA Removal Solution

Reagents G1 and G2 can be used until the expiration date indicated on the vials.

- Rehydrate the freeze-dried reagent G1 in 1 ml of *Legionella*-free distilled water or Milli-Q water for 5 to 10 min at room temperature. Gently mix by inverting the vial. **DO NOT VORTEX**
- After hydration, transfer G1 (1 ml) to the activation buffer vial (G2, 9 ml). Gently mix by inverting the vial

The solution is now activated and ready to use. You will need 40 µl of the activated solution (G1 + G2) for each sample.

Treatment with the Activated Solution (G1 + G2)

The Aquadien Free DNA Removal Solution can be used as part of the Aquadien Short DNA Extraction Protocol (bulletin 7021).

- Filter the water sample
- Add the filter in a tube containing 460 µl *Legionella* DNA-free water and 40 µl FDRS
- Invert the tube up and down for homogenization (do not vortex)
- Incubate at 37°C for 30 min
- Add 500 µl R1 to inactivate FDRS and for DNA extraction
- Vortex for 10 sec
- Incubate for 15 min at 95°C and 1,300 rpm in a shaking heating block
- Carefully take out the filter by pressing it on the walls of the tube to recover all the solution
- Centrifuge at 900 x g for 3 min
- Place the purification column in a collector vial
- Apply 500 µl of the supernatant on the purification column
- Centrifuge at 6,000 x g for 10 min
- Add 100 µl of R2 solution and throw away the collector vial
- Cover the purification column with a clean collector vial and turn both upside down
- Centrifuge at 1,000 x g for 3 min
- Throw away the purification column; 100 µl of purified DNA is obtained
- Use 5 µl of the extracted DNA for real-time PCR analysis

B. iQ-Check *Legionella* Protocols

Please refer to the product inserts for iQ-Check *Legionella* Kits (Bio-Rad catalog #3578102–3578105).

Technical Support in the United States

For technical assistance in the U.S., please call 1-800 4BIORAD (424-6723). Select option 2 for technical support. To place an order, please call 1-800 4BIORAD and press option 1 for customer service.

Visit foodscience.bio-rad.com and bio-rad.com/water for more information about Bio-Rad's food and water testing products.

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