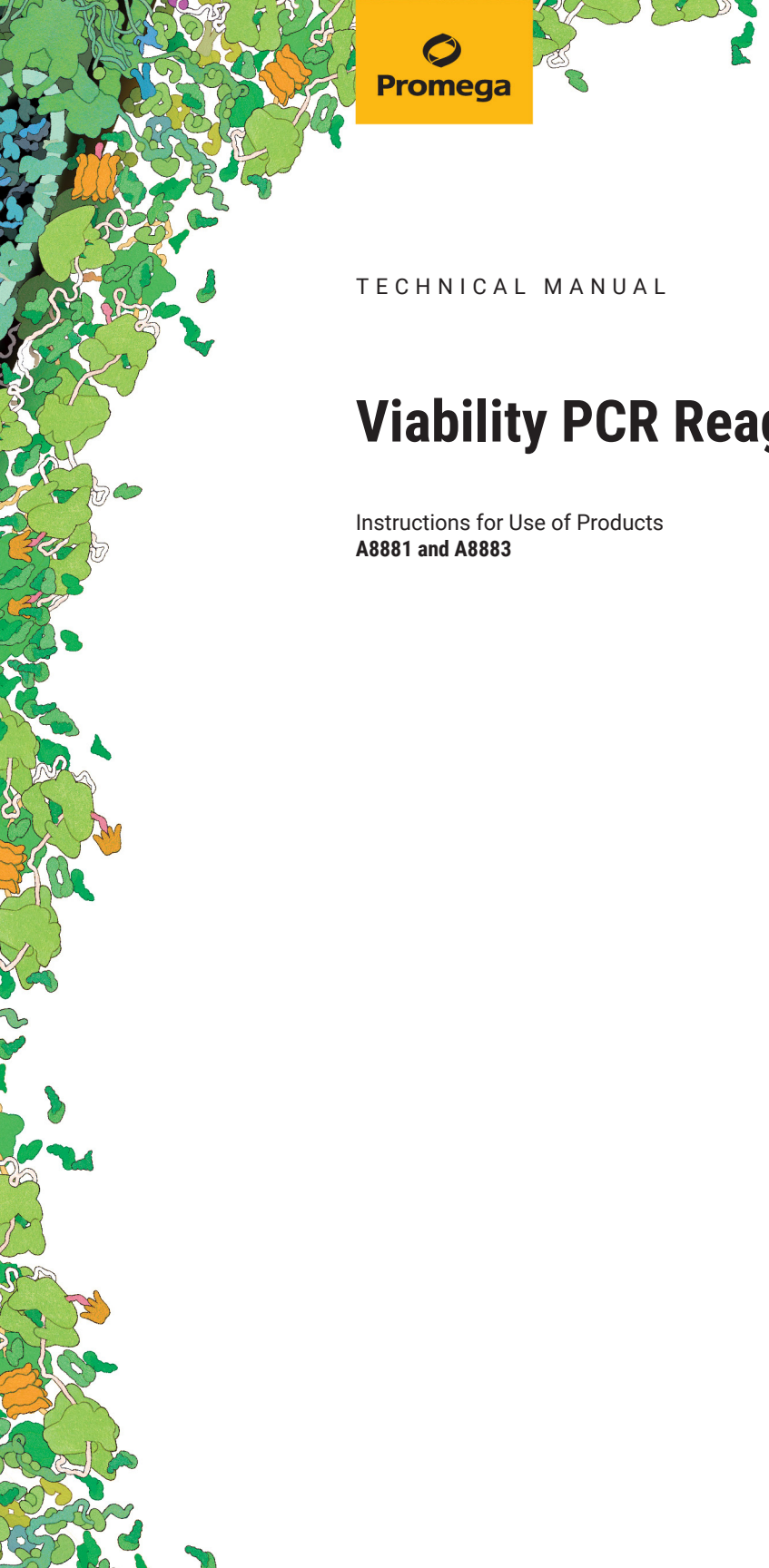

TECHNICAL MANUAL

Viability PCR Reagent System

Instructions for Use of Products
A8881 and A8883

11/24
TM736

Viability PCR Reagent System

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Culture-based detection is commonly used worldwide as part of routine microbial surveillance. Despite this widespread acceptance, culture-based approaches are limited in time-to-answer and lack the ability to detect "viable but nonculturable" (VBNC) bacteria that are often residually present after exposure to environmental stressors (antibiotics, nutrient deprivation, disinfectants, etc.). Culture-based approaches to measure viral viability are often expensive, cumbersome and technically challenging. PCR-based detection directly addresses these issues and is quickly becoming a widely adopted method for molecular-based assays.

The main drawback of existing PCR approaches is the lack of live-dead discrimination in the analysis of test results. The Viability PCR Reagent System combines the rapid results of PCR and the ability to discriminate between signal from viable and nonviable cells and viruses. This kit contains a cell- and capsid-impermeable intercalating reagent that exclusively binds to nucleic acid from membrane- or capsid-compromised nonviable cells and viruses. Once bound, the molecule irreversibly and covalently modifies nucleic acid, making it nonamplifiable in PCR. As a result, the signal from the PCR detection reaction results almost exclusively from viable cells or viruses. The Viability PCR Reagent concept is summarized in Figures 2 and 3.

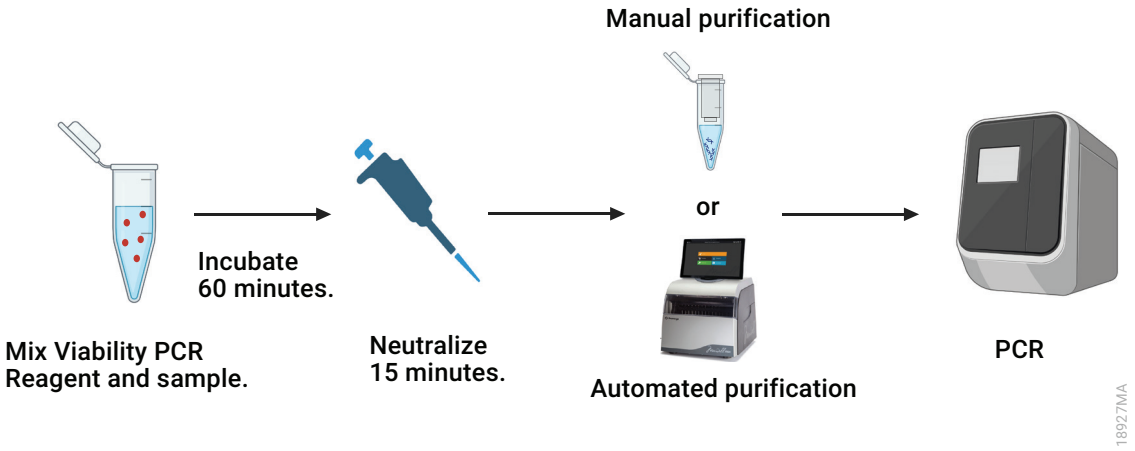

Figure 1. Viability PCR Reagent workflow. Viability PCR Reagent is added to sample and incubated at 37°C for 60 minutes, then neutralized for 15 minutes. Nucleic acid is purified and PCR performed. (Created with BioRender.com.)

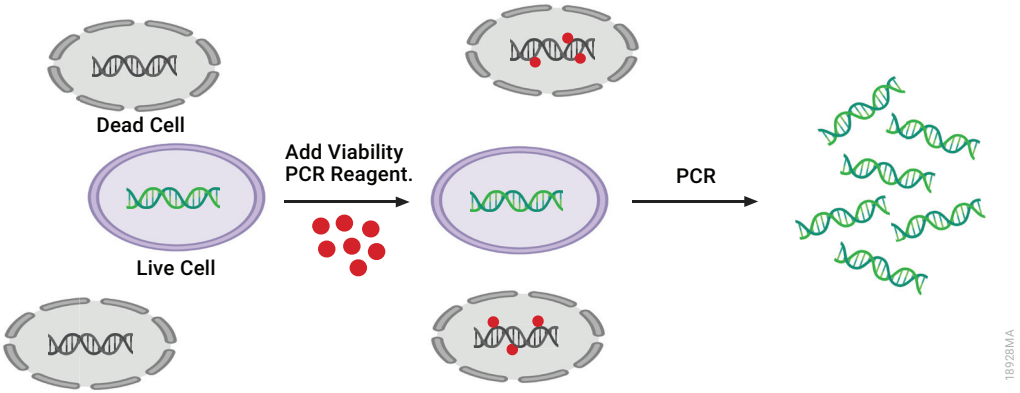

Figure 2. Live-dead discrimination with bacteria. Viability PCR Reagent is added to the sample and is impermeant to cells with intact membranes (purple cells). In contrast, the Viability PCR Reagent penetrates cells with compromised membranes (gray cells) and covalently modifies DNA. As a result, a PCR signal is only observed from viable cells. (Created with BioRender.com.)

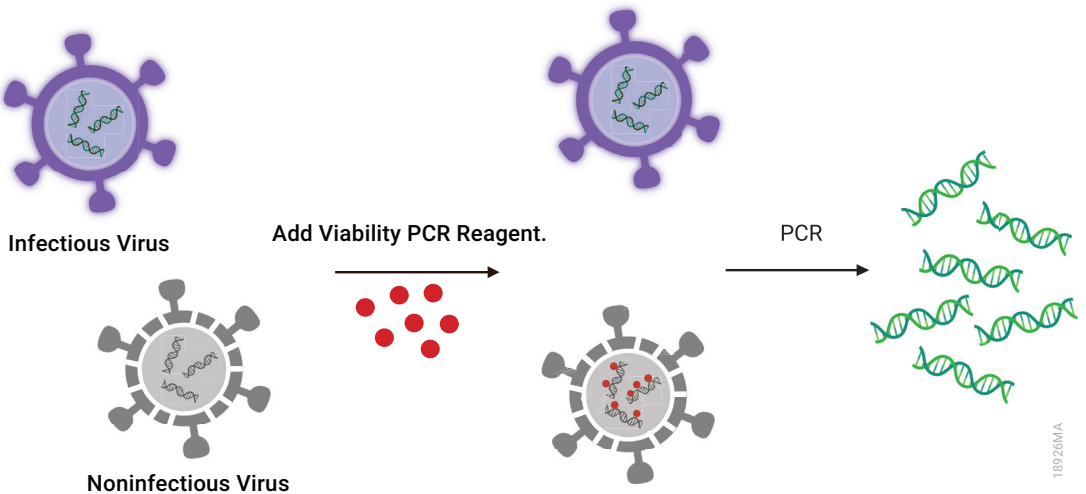

Figure 3. Intact-nonintact discrimination with viruses. Viability PCR Reagent is added to sample and is excluded by viruses with intact capsids (purple). In contrast, the Viability PCR Reagent (red dots) penetrates viruses with compromised capsids (gray) and covalently modifies nucleic acid. As a result, the PCR signal is only observed from intact viruses. (Created with BioRender.com.)

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Viability PCR Reagent System	**100 reactions**	**A8881**

Includes:

- 1 × 250µl Viability PCR Reagent (1mM)
- 1 × 5ml Viability PCR Neutralization Buffer (10X)

PRODUCT	SIZE	CAT.#
Viability PCR Reagent System, High Concentration	**100 reactions**	**A8883**

Includes:

- 1 × 50µl Viability PCR Reagent (30mM)
- 2 × 5ml Viability PCR Neutralization Buffer (10X)

Storage Conditions: Store the kit components at –30°C to –10°C protected from light.

Safety Note: Proper laboratory personal protective equipment (gloves, lab coat, safety glasses) should be used while handling the Viability PCR Reagent to reduce direct exposure risk. See the Safety Data Sheet (SDS) document for more information.

3. Viability PCR Reagent Protocol

Note: Reagent volumes can be scaled up or down as desired depending on the sample volume.

Materials to Be Supplied By the User

- 1.5ml microcentrifuge tubes
- heat block or incubator
- static incubator
- vortex mixer
- 1X PBS (phosphate-buffered saline)

3.A. Preparing Working Solution

Viability PCR Reagent treatment concentrations may need to be optimized for the specific sample type and test setup. Modify the reagent volume added to reactions as needed. If you need working reagent stocks with lower concentrations, dilute the reagent using 1X PBS. Dispose of any diluted reagent that is not used within several hours, as it is not suitable for long-term storage.

3.B. Adding Viability PCR Reagent

The final concentration of Viability PCR Reagent used in this protocol is 10µM.

Notes:

a. Viability PCR Reagent treatment concentrations may need to be optimized for the specific bacteria or virus and sample type being treated. Modify the reagent volume added to reactions as needed. Suggested treatment concentrations are 1–50µM.

b. The volume of sample used in the Viability PCR Reagent protocol can be altered. Modify the reagent volume and neutralization buffer added to reactions as needed, maintaining a 1:10 ratio of Neutralization Buffer:sample.

c. Viability PCR Reagent treatment incubation time at 37°C may need to be optimized for the specific bacteria or virus and sample type being treated. Suggested treatment time is 60–90 minutes.

d. Thaw Viability PCR Reagent at room temperature before use. Make sure reagent is completely thawed and vortex before use.

Caution: A Viability PCR Reagent concentration that is too high may inhibit live bacterial cell/intact virus qPCR signal. Viability PCR Reagent treatment should be optimized using live bacteria/intact virus as a baseline. Optimal treatment concentration should not significantly inhibit live bacteria or intact virus PCR signal.

1. Add 200µl of each test sample to two separate 1.5ml microcentrifuge tubes.
2. Add 2µl of 1mM Viability PCR Reagent to one tube (10µM final concentration; "+VR"), omitting the Viability PCR Reagent from other tube ("–VR"). Vortex to mix. Incubate at 37°C for 30 minutes.
3. Vortex to mix, then incubate at 37°C for an additional 30–60 minutes.
4. Add 20µl of Viability PCR Neutralization Buffer (10X) to each tube. Vortex to mix.
5. Incubate at room temperature for 15 minutes.

3.C. Nucleic Acid Purification

A variety of manual or automated nucleic acid purification workflows (see Section 7) can be used to isolate sample nucleic acid after the Viability PCR Reagent treatment and neutralization. Please visit: **www.promega.com/products/nucleic-acid-extraction/** or contact Technical Services: **techserv@promega.com** for more information about potential purification solutions for a variety of sample types.

4. PCR Protocol

Follow a real-time PCR (qPCR) , reverse transcriptase real-time PCR (RT-qPCR) or digital PCR (dPCR) protocol after treatment with Viability PCR Reagent and purification of nucleic acid.

A variety of amplicon sizes can be used, but we recommend amplicon lengths of >200bp to optimize effectiveness of the Viability PCR Reagent protocol.

5. Data Analysis

The difference in qPCR cycle threshold (C_q) values between samples treated with Viability PCR Reagent (VR) and not treated (–VR), is known as the ΔC_q value.

This value can be used in the equation below to calculate the percentage of viable bacteria or viruses in the sample.

$$\text{Percent intact virus or bacteria} = \frac{1}{2^{[\text{Cq (with VR)} - \text{Cq (without VR)}]}} \times 100$$

An optimized viability qPCR assay will maximize the ΔC_q value without significantly changing the C_q value of a viable control.

In a well-optimized assay the +VR treatment should result in qPCR signal almost exclusively from viable cells. In the example figure below, a large difference is observed between sample treated with and without viability reagent. This indicates that many nonviable cells or ruptured viral particles could be present in this sample.

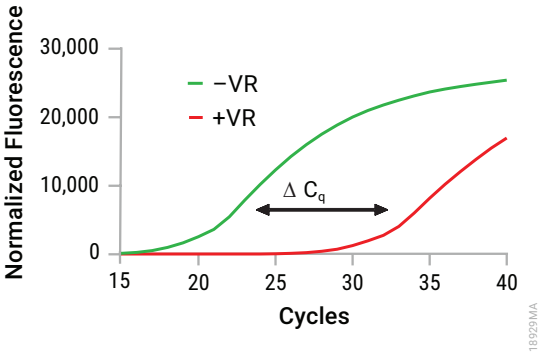

Figure 4. General representation of ΔC_q value as used in viability PCR (vPCR) experiments.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Significant suppression of viable bacterial or intact viral PCR signal	Reduce final concentration of Viability PCR Reagent added to samples.
	If using high concentration (30mM) Viability PCR Reagent stock, ensure that working solutions are diluted into PBS or water. DMSO can artificially permeabilize live cells and viruses.
	If resuspending or washing samples, make sure to use an isotonic buffer (e.g., PBS). Millipore® or deionized water comprises a hypotonic environment that can cause cell or capsid lysis or leakage.
	Ensure that the correct amount of Viability PCR Neutralization Buffer is added to the sample before nucleic acid purification.
Insufficient reduction of nonviable/nonintact bacterial or viral PCR signal when treating with Viability PCR Reagent (small ΔC_q value)	Increase the final concentration of Viability PCR Reagent added to samples.
	If possible, dilute complex sample 1:10 before treatment with Viability PCR Reagent. Samples with a very high bioburden may reduce effectiveness of Viability PCR Reagent.
	If a filter treatment protocol is being used with a filter material other than polycarbonate, try alternative filter material.
	Ensure that aqueous working solutions of Viability PCR Reagent are discarded after use, as reagent stability in aqueous solution is not guaranteed.
Lack of PCR detection of bacteria in 200µl of suspension sample	Filter-concentrate a large volume of bacterial suspension sample before Viability PCR Reagent treatment. Please contact Technical Services (**techserv@promega.com**) for guidance with on-filter Viability PCR Reagent treatment protocols. See the *GoTaq*® Legionella *qPCR and vPCR Systems Technical Manual*, TM725, for details.

7. Related Products

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC PureWater System	48 preps	AS2110
Maxwell® RSC PureFood GMO and Authentication System	48 preps	AS1600
Maxwell® RSC PureFood Pathogen System	48 preps	AS1660
Maxwell® RSC RSC Whole Blood DNA System	48 preps	AS1520
Wizard® PureWater System	48 preps	A3130
GoTaq® *Legionella pneumophila* qPCR System	1 each	AM2201
GoTaq® *Legionella spp/pneumophila*/SG1 qPCR System	1 each	AM2202
GoTaq® *Legionella pneumophila* Viability qPCR System	1 each	AM2205
GoTaq® *Legionella spp/pneumophila*/SG1 Viability qPCR System	1 each	AM2206

[a]Patent Pending.