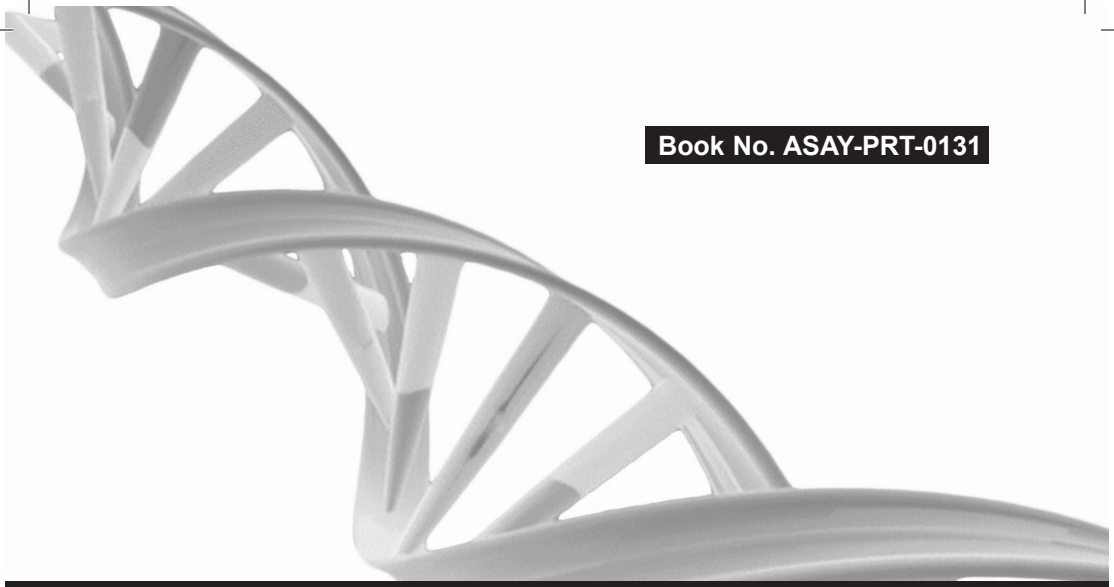

Book No. ASAY-PRT-0131

# *Freeze-Dried Reagent*

## *Detection Kit Instruction Booklet*

**For Hybridization Probe Assays**

BIOFIRE® DEFENSE

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Hybridization Probe Freeze-Dried Reagent Instruction Booklet ASAY-PRT-0131-03 01/17



This product is for presumptive testing only, and is not to be used for diagnosis of disease. Positive tests should be confirmed by a certified public health laboratory. Negative results are possible even when the target organism is present, due to various factors including PCR inhibitors and improper sample preparation, damage to the test kit or instrument, or human error.

# Table of Contents

## Introduction: Kit Contents

These reagents are for the identification of their described target agent. Use the accompanying assay card for specific information relating to the described target.

This instruction booklet describes procedures to perform real-time polymerase chain reaction (PCR) based assays on BioFire Defense's Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.®) Instruments. These procedures describe reagent setup and results analysis. For additional information, consult the system manual that accompanied the instrument and the accompanying assay card.

| **Kit Contents** | | |
|---|---|---|
| **Vial** | **Label** | **Contents and Use** |
| **Green Label** | Positive Control | Ready-to-use hybridization probe mix. Each vial contains reagents for two 20 µL reactions.<br>Contains purified amplicon for positive result. |
| **Red Label** | Negative Control | Ready-to-use hybridization probe mix. Each vial contains reagents for two 20 µL reactions. |
| **Blue Label** | Unknown | Ready-to-use hybridization probe mix. Each vial contains reagents for two 20 µL reactions. |
| **Amber Tube** | Reagent grade water | 2 x 850 µL |

| **Additional Equipment Needed** | |
|---|---|
| R.A.P.I.D. Instrument with laptop and current software | LightCycler® capillaries |

## General Safety Precautions

One of the most important rules of working in a laboratory environment is to avoid contamination: 1) use filter tips on your pipettes when working with any liquid solution, and 2) use different pipettes for setting up the reactions and for handling the samples.

# Laboratory Precautions

For general biosafety guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 4th Edition, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health (DHHS), May 1999. Available from http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

**Handling of Biohazard Wastes**
Dispose of used reagent vials in accordance with good laboratory practices. Waste from possible positive samples, or samples containing amplified DNA molecules, should be inactivated using appropriate procedures before disposal.

For more information refer to DHHS National Institute of Occupational Safety and Health (NIOSH) Publication No. 88-119, *Guidelines for Protecting the Safety and Health of Health Care Workers* (section 6, "Hazardous Waste Disposal").

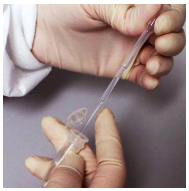
## Storage

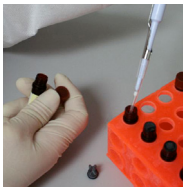
Store contents in a dry environment at room temperature (18–30 ºC). **DO NOT REFRIGERATE.** Do not allow the kit to sit in direct sunlight. When pouches are opened, use contents immediately. Discard unused reagents from open pouches. Reagents are stable through the expiration date printed on the label provided the storage recommendations have been followed. **Do not use reagents after they have expired.**

## Preventing Contamination During Setup

Some concerns for users of PCR based assays for detection are the sensitivity levels of the assay and the high copy number of product that is generated from the amplification. Both of these factors raise the risk of false positive calls, especially if a capillary is broken. To mitigate this risk of contamination, some important precautions can be taken. These precautions include collecting, purifying, preparing, and running a test in separate rooms or areas, as well as executing decontamination procedures after a capillary breakage occurs.

| Outside Sample Collection of Raw Sample | Lab Room / Area 1 Sample Purification | Lab Room / Area 2 Reaction Prep | Lab Room / Area 3 Run Reaction |
|---|---|---|---|





Start Finish

# Product Overview

This kit is specially adapted for amplification in glass capillaries using the R.A.P.I.D. Instrument and hybridization probes.

A fragment of the target DNA is amplified using specific primers. The amplicon is detected by fluorescence using a specific pair of hybridization probes. These probes consist of two different short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the reaction cycle. One probe is labeled at the 5' end with LCRed 640. To avoid extension on the 3' end, it is modified by phosphorylation. The second probe is labeled at the 3' end with fluorescein. Only after hybridization to the template DNA do the two probes come in close proximity, resulting in fluorescence resonance energy transfer (FRET) between the two fluorophores. The fluorescence emitted by the LCRed 640 dye is measured in channel 2 of the R.A.P.I.D. Instrument.

The fluorescent signal from the unknown sample is compared to the signals from the positive and negative control samples.

## Basic Stages of the Protocol

Amplify and monitor online in real-time for the presence of the target DNA.

**Step 1**: Purify and mix DNA with freeze-dried reagents and water
**Step 2**: Test samples using the R.A.P.I.D. Instrument
**Step 3**: View results with the auto-call software

**Note:** BioFire Defense strongly recommends using established DNA purification procedures using their IT 1-2-3™ DNA Purification Kits and a Vortex-Genie® 2 with 2 mL Turbomix® adapter.

**Assay Time**
Based on an assay for 2 positive, 2 negative, and 28 unknown samples:

| Procedure | Time |
|---|---|
| Set up test | 15 min. |
| Run test using R.A.P.I.D. Instrument | 25 min. |
| **Total Experiment Time** | **40 min.** |

**Capillary Handling**
Never touch the reaction capillaries with bare hands. Always wear gloves when handling capillaries.

## Reagent Setup

Before preparing reagents, obtain the appropriate numbers of Negative Control (NC), Positive Control (PC), and Unknown reagent vials, as well as Reagent Grade Water. Each reagent vial contains enough master mix for two reactions. Freeze-dried reagent vials contain all of the components required for PCR. Negative and Unknown vials lack DNA template and are used to setup NC and Unknown samples. PC vials contain reagents that have been freeze-dried with specified quantities of target template DNA.

Reactions should be prepared so that the NC samples are set up first, then the Unknown samples, and lastly the PC samples. Preparation in this order will reduce the potential of contaminating the Unknown samples with PC template.

## Negative Controls

Put on a clean pair of gloves. Prepare the freeze-dried NC samples as follows:

1. To ensure that the reagent pellet or pellet fragments are at or near the bottom of the vial, tap the NC reagent vial on the bench or centrifuge for 3 sec.
2. Remove the rubber cap from each NC reagent vial.

3. Visually check that the reagent pellet is at or near the bottom of the vial. If not, with a clean pipette tip, push the pellet or fragment(s) to the bottom of the vial. Change the pipette tip.
4. To the NC reagent vial, add **40 µL** of **Reagent Grade Water**. Change the pipette tip.
5. Replace cap on each reagent vial.
6. Vortex the NC reagent vial for 5 sec at maximum speed.
7. Load reagent vial in minicentrifuge and centrifuge for 3 sec to bring all liquid to the bottom of the vial.
8. Visually check that the entire pellet has been rehydrated. If not repeat steps 6 through 8.
9. Pipette **19 µL** of the **hydrated mixture** into a capillary. Repeat pipette step into a second capillary to obtain a duplicate.
10. Cap capillaries and discard gloves. Record capillary number, sample type, and assay name. Change the pipette tip.

## Unknown Samples

Put on a clean pair of gloves. Prepare freeze-dried Unknown samples as follows:

**Note:** To minimize the risk of cross-contamination, each unknown sample should be processed from steps 1 through 11 before another unknown sample is set up.

1. To ensure that the reagent pellet or pellet fragments are at or near the bottom of the vial, tap the Unknown reagent vial on the bench or centrifuge for 3 sec.
2. Remove the rubber cap from the Unknown reagent vial.
3. Visually check that the reagent pellet is at or near the bottom of the vial. If not, with a clean pipette tip, push the pellet or fragment(s) to the bottom of the vial. Change the pipette tip.
4. To the Unknown reagent vial, add **40 µL** of **Sample**.
6. Replace cap on reagent vial.
7. Vortex the Unknown reagent vial for 5 sec at maximum speed.
8. Load Unknown reagent vial in minicentrifuge and centrifuge for 3 sec to bring all liquid to the bottom of the vial.
9. Visually check that the entire pellet has been rehydrated. If not repeat steps 6 through 8.
10. Pipette **19 µL** of the **hydrated mixture** into a capillary. Repeat pipette step into a second capillary to obtain a duplicate. Change the pipette tip.

11. Cap capillaries and discard gloves. Record capillary number, sample information, and assay name.

## Positive Controls

⚠ **WARNING:** High risk of cross-contamination. Follow instructions carefully.

Put on a clean pair of gloves. Prepare freeze-dried PC samples as follows:

1. To ensure that the reagent pellet or pellet fragments are at or near the bottom of the vial, tap the PC reagent vial on the bench or centrifuge for 3 sec.
2. Remove the rubber cap from each PC reagent vial.
3. Visually check that the reagent pellet is at or near the bottom of the vial. If not, with a clean pipette tip, push the pellet or fragment(s) to the bottom of the vial. Change the pipette tip.
4. To the PC reagent vial, add **40 µL** of **Reagent Grade Water**. Change the pipette tip.
5. Replace cap on reagent vial.
6. Vortex the PC reagent vial for 5 sec at maximum speed.
7. Load reagent vial in minicentrifuge and centrifuge for 3 sec to bring all liquid to the bottom of the vial.
10. Visually check that the entire pellet has been rehydrated. If not repeat steps 6 through 8.
11. Pipette **19 µL** of the **hydrated mixture** into a capillary. Repeat this step into a second capillary to obtain a duplicate.
12. Cap capillaries and discard gloves. Record capillary number, sample type, and assay name. Discard pipette tip.

## Centrifuging Capillaries

1. Once all of the reagents have been prepared and transferred to capillaries, load capillaries in a minicentrifuge fitted with capillary adaptors.
2. Centrifuge at the **lowest speed setting** by holding down the pulse button for 3 sec. This transfers the liquid down to the tips of the capillaries and prepares them for testing.

⚠ **WARNING:** Centrifuging capillaries at full speed will cause them to break.

3. Once the samples are prepared, use the following protocol for your specific instrument. Please note especially that the denature temperatures are different between the R.A.P.I.D. 7200 and R.A.P.I.D. 9200. Follow the instructions found in the instrument users guide on loading the carousel and running a test.

## Amplification Procedures

**Starting the R.A.P.I.D. Software**
Refer to the R.A.P.I.D. System Manual for detailed instructions on setting up and using the R.A.P.I.D. software for an experiment.

**Experimental Protocol**
This protocol consists of three programs. Use the appropriate protocol for your instrument.

**Program 1:** Denaturation of the target DNA
**Program 2:** Amplification of the target DNA
**Program 3:** Melting of the target DNA

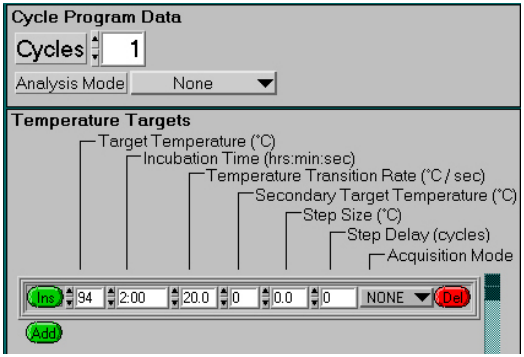
## Two Types of Tests

**Batch Tests**—Batch tests are used for a single assay on multiple samples. Follow the directions below to avoid cross-contamination.

**Screen Tests**—Screen tests are for multiple assays on a single or limited number of samples. To minimize the risk of cross-contamination, prepare all Unknown reactions from one purified sample and transfer to capillaries before beginning setup with another unknown sample. Next, prepare all NCs and transfer them to capillaries. Prepare all PCs last and transfer to capillaries to finish the reconstitution procedure.

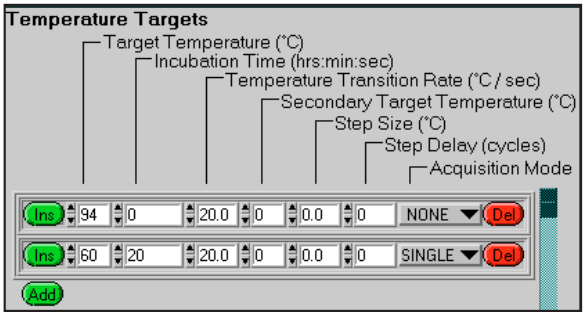
## R.A.P.I.D. 7200 Protocol (DNA)

The following protocol is for the R.A.P.I.D. 7200 Instrument. The image below illustrates where the protocol needs to be entered in the run software.




**Program 1: DNA Denaturation**

| Cycle Program Data | Value |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| **Temperature Targets** | **Segment 1** |
| Target Temperature | **94 ºC** |
| Incubation Time (hrs:min:s) | 120 s |
| Temperature Transition Rate | 20 ºC/s |
| Second Target Temperature | 0 ºC |
| Step Size | 0 ºC |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |



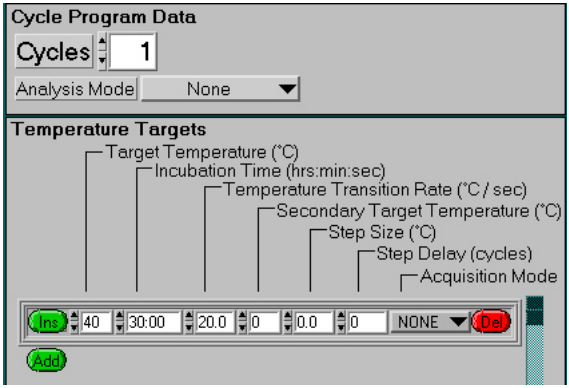

**Program 2: Amplification**

| Cycle Program Data | Value | |
|---|---|---|
| Cycles | 45 | |
| Analysis Mode | Auto Analysis | |
| **Temperature Targets** | **Segment 1** | **Segment 2** |
| Target Temperature | 94 °C | 60 °C |
| Incubation Time (hrs:min:s) | 0 s | 20 s |
| Temperature Transition Rate | 20 °C/s | 20 °C/s |
| Second Target Temperature | 0 °C | 0 °C |
| Step Size | 0 °C | 0 °C |
| Step Delay (Cycles) | 0 | 0 |
| Acquisition Mode | None | Single |

**Fluorescence Parameters**

**Display Mode:** fluorescence channel 2 (Ch2/1)
Set the fluorescence gains as follows:

| Fluorimeter Gain | Value |
|---|---|
| Channel 1 (F1) | 1 |
| Channel 2 (F2) | 8 |
| Channel 3 (F3) | 1 |




**Program 1: RNA Sample Preparation Hold**

| Cycle Program Data | Value |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| **Temperature Targets** | **Segment 1** |
| Target Temperature | **40 ºC** |
| Incubation Time (hrs:min:s) | 30 min |
| Temperature Transition Rate | 20 ºC/s |
| Second Target Temperature | 0 ºC |
| Step Size | 0 ºC |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

**Program 2: RNA Denaturation**

| Cycle Program Data | Value |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| **Temperature Targets** | **Segment 1** |
| Target Temperature | **94 ºC** |
| Incubation Time (hrs:min:s) | 120 s |
| Temperature Transition Rate | 20 ºC/s |
| Second Target Temperature | 0 ºC |
| Step Size | 0 ºC |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

**Program 3: RNA Amplification**

| Cycle Program Data | Value | |
|---|---|---|
| Cycles | 45 | |
| Analysis Mode | Auto Analysis | |
| **Temperature Targets** | **Segment 1** | **Segment 2** |
| Target Temperature | 94 ºC | 60 ºC |
| Incubation Time (hrs:min:s) | 0 s | 20 s |
| Temperature Transition Rate | 20 ºC/s | 20 ºC/s |
| Second Target Temperature | 0 ºC | 0 ºC |
| Step Size | 0 ºC | 0 ºC |
| Step Delay (Cycles) | 0 | 0 |
| Acquisition Mode | None | Single |

**Fluorescence Parameters**

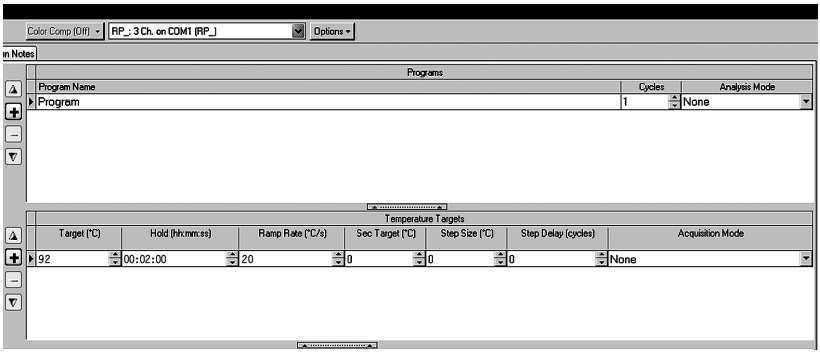
**Display Mode:** fluorescence channel 2 (Ch2/1)

Set the fluorescence gains as follows:

| Fluorimeter Gain | Value |
|---|---|
| Channel 1 (F1) | 1 |
| Channel 2 (F2) | 8 |
| Channel 3 (F3) | 1 |

## R.A.P.I.D. 9200 and LT Protocol (DNA)

The following protocol steps are for the R.A.P.I.D. 9200 and LT Instruments. The image below illustrates where the protocol needs to be entered.




**Program 1: DNA Denaturation**

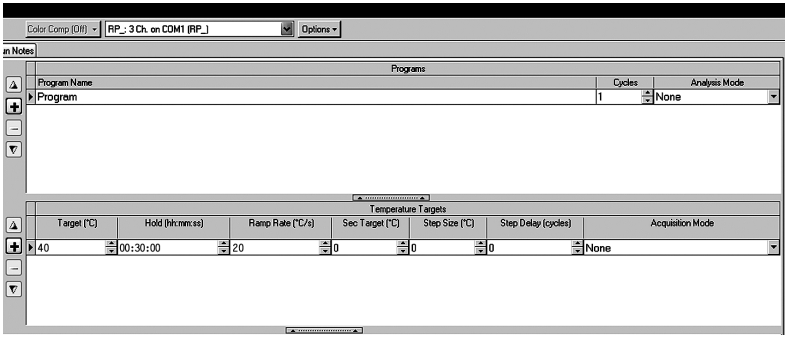
| Cycle Program Data | Value |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| **Temperature Targets** | **Segment 1** |
| Target | **92 ºC** |
| Hold (hh:mm:ss) | 120 s |
| Ramp Rate | 20 ºC/s |

| | |
|---|---|
| Sec Target | 0 ºC |
| Step Size | 0 ºC |
| Step Delay (cycles) | 0 |
| Acquisition Mode | None |

**Program 2: DNA Amplification**

| Cycle Program Data | Value | |
|---|---|---|
| Cycles | 45 | |
| Analysis Mode | Quantification | |
| **Temperature Targets** | **Segment 1** | **Segment 2** |
| Target | 92 ºC | 60 ºC |
| Hold (hh:mm:ss) | 0 s | 20 s |
| Ramp Rate | 20 ºC/s | 20 ºC/s |
| Sec Target | 0 ºC | 0 ºC |
| Step Size | 0 ºC | 0 ºC |
| Step Delay (cycles) | 0 | 0 |
| Acquisition Mode | None | Single |

## R.A.P.I.D. 9200 and LT Protocol (RNA)



**Program 1: RNA Sample Preparation Hold**

| Cycle Program Data | Value |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| **Temperature Targets** | **Segment 1** |
| Target Temperature | **40 ºC** |
| Incubation Time (hrs:min:s) | 30 min |
| Temperature Transition Rate | 20 ºC/s |
| Second Target Temperature | 0 ºC |
| Step Size | 0 ºC |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

**Program 2: RNA Denaturation**

| Cycle Program Data | Value |
|---|---|
| Cycles | 1 |
| Analysis Mode | None |
| **Temperature Targets** | **Segment 1** |
| Target Temperature | **92 ºC** |
| Incubation Time (hrs:min:s) | 120 s |
| Temperature Transition Rate | 20 ºC/s |
| Second Target Temperature | 0 ºC |
| Step Size | 0 ºC |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

**Program 3: RNA Amplification**

| Cycle Program Data | Value | |
|---|---|---|
| Cycles | 45 | |
| Analysis Mode | Auto Analysis | |
| **Temperature Targets** | **Segment 1** | **Segment 2** |
| Target Temperature | 92 ºC | 60 ºC |
| Incubation Time (hrs:min:s) | 0 s | 20 s |
| Temperature Transition Rate | 20 ºC/s | 20 ºC/s |
| Second Target Temperature | 0 ºC | 0 ºC |
| Step Size | 0 ºC | 0 ºC |
| Step Delay (Cycles) | 0 | 0 |
| Acquisition Mode | None | Single |

# Results

The results shown below are for R.A.P.I.D. Instrument models 7200, 9200, and LT. Use the appropriate instructions for your system.

**R.A.P.I.D. 7200**
A successful test yields results similar to the ones shown below. Results are displayed in grouped panels if multiple organisms were tested.

**Organism Present**

| # | Sample Name | Control | Score | Result |
|---|---|---|---|---|
| 1 | Sample 1 | Positive | 1148 | Present |
| 2 | Sample 2 | Positive | 1046 | Present |
| 4 | Sample 4 | Negative | 1 | Not Detected |
| 5 | Sample 5 | Negative | 2 | Not Detected |
| 6 | Sample 6 | Unknown | 214 | Present |
| 7 | Sample 7 | Unknown | 15 | Not Detected |
| 8 | Sample 8 | Unknown | 6 | Not Detected |
| 9 | Sample 9 | Unknown | 4 | Not Detected |

For each capillary, the results include the following:

- **#—**Identifies the carousel position of the capillary.
- **Sample Name—**Provides the name of the sample.
- **Control—**Identifies the sample type for each sample.
- **Score—**The score is derived by calculating the relationship between the sample's fluorescence and the level of background fluorescence in the sample.
- **Result—**Displays the overall result for the sample.

The possible results include the following:

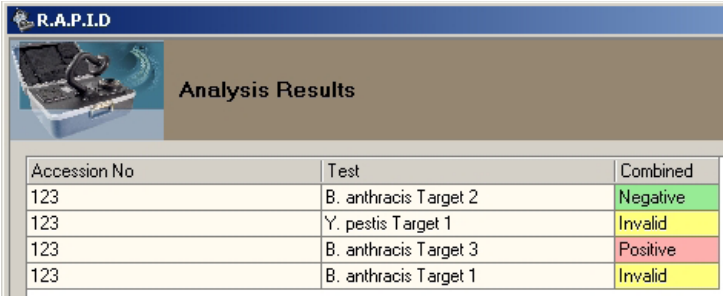
| | |
|---|---|
| **Present** | A red **Present** call for an unknown indicates that the target was identified in that sample and the controls were successful. A blue Present call is for a positive control. |
| **Not Detected** | A green **Not Detected** call for an Unknown indicates that the target was not identified in that sample and the test controls were successful. A blue Not Detected call is for a negative control. |
| **Please Repeat** | A **Please Repeat** indicates that the positive or negative control failed. |

**Positive** and **Please Repeat** results should be referred to a supervisor or advanced operator for appropriate follow-up.

To display the real-time fluorescence data for each sample, select the sample of interest and click the **Show Graph** button at the bottom of the screen. For more detailed information on performing a test and analyzing the results, see the *R.A.P.I.D. System Manual* that came with your instrument.

**R.A.P.I.D. 9200 and LT**

A successful test yields results similar to the ones shown below. Results are displayed in grouped panels if multiple organisms were tested.




For each sample, the results include the following:

- **Accession No.**
- **Test—**pathogen target
- **Combined Result—**is a combination of the control results and the unknown results. See chart below
- **Concentrations—**quantification results for certain positive samples (specific target/specimen combinations) including appropriate units.

The Results window displays results only for the capillaries that contain unknown samples.

The possible results include the following:

| | |
|---|---|
| **Positive** | A red positive call indicates that the target was identified in that sample and the run controls were successful. A sample that is detected as positive will generate an audible signal in the software. |
| **Negative** | A green negative call indicates that the target was not identified in that sample and all the run controls (NC and PC) were successful. |
| **Uncertain** | A yellow uncertain call indicates that the instrument was unable to interpret data and the controls were successful. |
| **Invalid** | A yellow invalid call indicates that a PC or NC failed. All samples connected to that control would be invalid. |

**Positive, Invalid,** and **Uncertain** results should be referred to a supervisor or advanced operator for appropriate follow-up.

Click **Finish** to view a report of the test results. For more detailed information on performing a test and analyzing the results, see the *R.A.P.I.D. System Manual* that came with your instrument.

**Note:** Melting curve temperatures can be affected by final DNA concentration as well as contaminants like residual ethanol from DNA preparation. Temperatures are approximate and can shift depending on experimental variables.

# Appendix A: Decontamination

The list below provides items that are necessary in a laboratory for decontamination purposes.

- 10% bleach solution in a squeeze or spray bottle
- Water in a squeeze or spray bottle
- 95% ethanol in a squeeze bottle
- DNA*Zap*™ or equivalent DNA degrading system
- Paper towels
- Nylon brush
- Bleach wipes
- Lens cleaner
- Lens paper

## Broken Capillary

**WARNING**: Immediately after capillary breakage make sure no one uses any potentially contaminated areas or instruments. Follow the steps below to properly decontaminate and dispose of the capillary.

1. Put on PPE such as gloves and safety glasses.
2. Stop the run and unplug the instrument before beginning cleanup.
3. Ensure no one else uses any potentially contaminated areas or instruments.
4. Decontaminate and dispose of the broken capillary using the following steps:
    a. Dispose of potentially contaminated gloves.
    b. Put on a clean pair of gloves and dispose of the potentially contaminated lab coat.

c. Clean up broken glass, discard in capillary containment bin or biohazardous waste container, and complete removal of capillaries from carousel (if necessary).
d. Change gloves.
e. Follow decontamination procedures for cleaning affected area.

## Sample Carousel Decontamination

R.A.P.I.D. 7200 carousels are custom fitted to each instrument. R.A.P.I.D. 9200 and LT carousels are interchangeable and disposable. Contact BioFire Defense to obtain a replacement carousel if the decontamination procedures fail to eliminate all contamination or the carousel is damaged.

1. Place carousel on bench top.
2. Spray 10% bleach solution on top and bottom of carousel. Let sit for 5–10 min.
3. Wipe carousel and bench area with clean paper towel.
4. Repeat steps 2 and 3 twice, for a total of three wipes.
5. Wipe carousel and bench area with new paper towel.
6. Spray distilled water on both sides of carousel.
7. Wipe carousel and bench area; dry with a new paper towel.
8. Spray both sides of carousel with DNA*Zap*, or equivalent product. See product for proper usage.
9. Rinse by spraying distilled water on both sides of carousel.
10. Wipe carousel and bench area dry with a new paper towel. Tap the carousel on your hand or clean bench top to remove excess distilled water.
11. Use a squirt bottle of 95% ethanol to cover the entire carousel with ethanol. In particular, squirt ethanol into the capillary holes to remove any excess bleach.
12. Wipe carousel and bench area dry with a new paper towel. Tap the carousel on your hand or clean bench top to remove excess ethanol.
13. Use a new nylon brush to dry out all holes in the carousel. Make sure carousel is dry, as residual bleach could alter fluorescence readings.* If desired, check all carousel holes by inserting a clean, empty capillary. If insertion and removal of a capillary is difficult for any hole, repeat rinsing and drying steps.
14. Discard nylon brush.

* Due to brass components, any bleach that is not completely rinsed away can cause corrosion. This is in turn can cause increased friction leading to broken capillaries and contamination in future runs. BioFire Defense recommends replacing disposable carousels (models 9200 and LT). Custom-fitted carousels (model 7200) must be thoroughly rinsed and dried.

## Instrument Decontamination

1. Remove carousel from machine and follow carousel decontamination procedures.
2. Remove broken glass from instrument (including the carousel chamber) with lens paper lightly moistened with water. You may carefully remove glass from the optics with the lens paper.
3. To remove glass from under the chamber fan, remove the fan blade according to the instrument manual.
4. Change gloves and wet a paper towel with 10% bleach and wipe the entire instrument (including the bottom of the instrument). Do not wipe bleach on the optics.
5. Repeat bleach step with a fresh paper towel and clean gloves.
6. Wet a paper towel with water and wipe the entire instrument (including the bottom of the instrument). Do not wipe the optics.
7. Repeat water step with a fresh paper towel.
8. Clean the optics of the instrument with lens cleaner and lens paper. Use light pressure when cleaning the optics.
9. Repeat optics cleaning step with a fresh lens paper.
10. Make a visual check of the thermocouple to make sure it is straight and not bent to one side or the other. This will ensure the thermocouple will not break the next carousel of capillaries. In the 7200, you can check the thermocouple alignment by placing the white storage carousel in the carousel chamber, making sure to align the notch in the storage carousel with the thermocouple.
11. Dispose of gloves and other potentially contaminated items.

## Decontamination for Other Areas

1. Spray 10% bleach solution on area of potential contamination. Let stand for 5 min.
2. Wipe with paper towel.
3. Repeat steps 1 and 2 twice, for a total of three wipes.
4. Spray area with distilled water.
5. Wipe dry with new paper towel.
6. Spray area with DNA*Zap*, or equivalent product. See product for proper usage.
7. Rinse by spraying area with distilled water and wiping dry.

# Appendix B: Troubleshooting

**Problem:** *No amplifications can be monitored.*

1. **Possible Cause—**Wrong channel chosen for real-time monitoring
   **Recommendation—**Check that the fluorescence channel selected is Channel 2.
2. **Possible Cause—**Measurements do not occur.
   **Recommendation—**Make sure “single” is selected as acquisition mode in programming screen.
3. **Possible Cause—**Inhibitory effects of sample material.
   **Recommendation—**Purify DNA sample using recommended kits.

**Problem:** *Fluorescence intensity too high.*

**Possible Cause—**Unsuitable gain settings.
**Recommendation—**Check gain settings in the programming screen. Lower settings if necessary.

**Problem:** *Fluorescence intensity too low.*

1. **Possible Cause—**Unsuitable gain settings.
   **Recommendation—**Check gain settings in the programming screen. Raise settings if necessary.
2. **Possible Cause—**Reaction mix exposed to light.
   **Recommendation—**Be sure to protect reaction vials from light. Try a new batch of reagents.
3. **Possible Cause—**Very low starting DNA.
   **Recommendation—**Use more sample DNA.

**Problem:** *Negative control samples are positive.*

**Possible Cause—**Contamination.
**Recommendation—**Replace all critical solutions.
Decontaminate pipettors.
Use aerosol free tips.
Use a fresh tip for each pipetting step.

# Appendix C: Melt Protocol

The following melt protocols are used to confirm test results.

## R.A.P.I.D. 7200 Melt Protocol

| Cycle Program Data | Value | | |
|---|---|---|---|
| Cycles | 1 | | |
| Analysis Mode | Melting Curves | | |
| **Temperature Targets** | **Segment 1** | **Segment 2** | **Segment 3** |
| Target Temperature | 94 ºC | 45 ºC | 80 ºC |
| Incubation Time (hrs:min:s) | 2 s | 140 s | 0 s |
| Temperature Transition Rate | 20 ºC/s | 20 ºC/s | 0.2 ºC/s |
| Second Target Temperature | 0 ºC | 0 ºC | 0 ºC |
| Step Size | 0 ºC | 0 ºC | 0 ºC |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | None | Step |

## R.A.P.I.D. 9200 and LT Melt Protocol

| Cycle Program Data | Value | | |
|---|---|---|---|
| Cycles | 1 | | |
| Analysis Mode | Melting Curves | | |
| **Temperature Targets** | **Segment 1** | **Segment 2** | **Segment 3** |
| Target Temperature | 92 ºC | 45 ºC | 80 ºC |
| Incubation Time (hrs:min:s) | 2 s | 140 s | 0 s |
| Temperature Transition Rate | 20 ºC/s | 20 ºC/s | 0.2 ºC/s |
| Second Target Temperature | 0 ºC | 0 ºC | 0 ºC |
| Step Size | 0 ºC | 0 ºC | 0 ºC |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | None | Step |

**Fluorescence Parameters**

**Display Mode:** fluorescence channel 2 (Ch2/1)

Set the fluorescence gains as follows:

| Fluorimeter Gain | Value |
|---|---|
| Channel 1 (F1) | 1 |
| Channel 2 (F2) | 8 |
| Channel 3 (F3) | 1 |

# Appendix D: Ordering Information

For additional product information visit us online at **www.BioFireDefense.com**.

## Freeze-Dried Reagent Kits

| Item | Part Number |
|---|---|
| BioThreat Screening Kit<br>Tests for: *B. anthracis* (Target 1), *F. tularensis* (Target 1), *Y. pestis* (Target 1), *Brucella species* (Target 1) | 3833 |
| Pathogen test Kit<br>Tests for: *Listeria monocytogenes* (Target 1), *E. coli* O157 (Target 1) , *Salmonella* species (Target 1), *Campylobacter* species (Target 1) | 3834 |
| Reagent Training Kit<br>Freeze-dried reagents used for training on the R.A.P.I.D. instrument. | 3832 |
| *B. anthracis* (Anthrax, Target 1) | 3828 |
| *B. anthracis* (Anthrax, Target 2) | 3841 |
| *B. anthracis* (Anthrax, Target 3) | 3887 |
| *F. tularensis* (Tularemia, Target 1) | 3830 |
| *F. tularensis* (Tularemia, Target 2) | 3882 |
| *Y. pestis* (Plague, Target 1) | 3831 |
| *Y. pestis* (Plague, Target 2) | 3881 |
| *Brucella species* (Brucellosis, Target 1) | 3829 |
| *Listeria monocytogenes* (Listeriosis, Target 1) | 3827 |
| *E. coli O157* (Target 1) | 3825 |
| *Salmonella* species (Salmonellosis, Target 1) | 3826 |

| Item | Part Number |
|---|---|
| *Campylobacter* species (Target 1) | 3839 |
| *C. botulinum* Type A (Botulism, Target 1) | 3840 |
| *Cryptosporidium* (Target 2) | ASAY-ASY-0101 |
| Variola (Small Pox) | ASAY-ASY-0102 |
| Ricin Target 1 | ASAY-ASY-0103 |
| Ricin Target 2 | ASAY-ASY-0104 |
| Avian Influenza H5 Subtype Target 1 | ASAY-ASY-0105 |
| Avian Influenza H5 Subtype Target 2 | ASAY-ASY-0106 |
| Influenza A Target 1 | ASAY-ASY-0109 |

## Sample Purification Kits and Supplies

| Item | Contents | Part Number |
|---|---|---|
| IT 1-2-3™ PLATINUM Path Sample Purification Kit | Supplies to purify 40 samples from a wide array of samples. | ASAY-ASY-0120 |
| IT 1-2-3™ SWIPE Sample Purification Kit | Supplies to purify 40 samples, 18 reactions per sample | ASAY-ASY-0005 |
| IT 1-2-3™ FLOW Sample Purification Kit | Supplies to purify 40 samples, 70 reactions per sample, includes protease | ASAY-ASY-0004 |
| IT 1-2-3™ SCOOP Sample Purification Kit | Supplies to purify 50 samples, 18 reactions per sample | ASAY-ASY-0502 |
| ITI 1-2-3™ VIBE Sample Purification Kit | Supplies to purify 40 samples, 9–18 reactions per sample, includes protease | ASAY-ASY-0500 |
| ITI 1-2-3™ RNA Module | Carrier RNA | ASAY-ASY-0501 |
| Filtered Blender Bags | 1 Bag of 10 (Brinkmann) | ASAY-ASY-0060 |
| PBS packets | Phosphate Buffered Saline, pH 7.4 PBS powder, 10 packets per box (each packet makes 1 L PBS) (Sigma-Aldrich) | ASAY-ASY-0061 |
| Triton X-100 | 100 mL Triton (Sigma-Aldrich) | ASAY-ASY-0062 |
| Stool Collection Vials (Cary Blair) | Box of 20 (PROTOCOL-Fisher) | ASAY-ASY-0063 |
| Stool Concentrator Filters | Box of 30 (PARAPAK, Meridian Diagnostics) | ASAY-ASY-0064 |

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