

Kit Part No: ASAY-ASY-0005

IT *1-2-3*[™] SWIPE

Sample Purification Kit

Instruction Booklet



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Abbreviations and Acronyms

BFDf.....	BioFire Defense
BMBL.....	Biosafety in Microbiological and Biomedical laboratories
BW.....	Biological Warfare
C.....	Celsius
DFU.....	Dry Filter Unit
DHHS.....	Department of Health and Human Services
DNA.....	Deoxyribonucleic acid
<i>g</i>	Gravity (= RCF)
LOD.....	Limit of Detection
mL.....	Milliliter
MSDS.....	Material Safety Data Sheet
PBS.....	Phosphate-buffered saline
RCF.....	Relative Centrifugal Force (= <i>g</i>)
RNA.....	Ribonucleic acid
μL.....	Microliter (0.000001 Liters)

General Safety Precautions

Laboratory Procedures and Precautions

- To avoid contamination, we recommend using filter tips on pipettes when working with any liquid solution.
- A Biosafety cabinet should be used when a potentially infectious material is used in procedures where there is potential for creating aerosols or splashes.
- When working with potentially harmful samples or chemicals always wear the appropriate personal protective equipment (lab coat, gloves, and eye protection).
- Avoid exposure to any potentially infectious samples or harmful chemicals. Exposure can occur by inhalation, ingestion or skin absorption.
- For more information on kit components consult the appropriate SDS provided by BioFire Defense.

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, May 1999. Available from <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm>

Handling of Biohazard Wastes

Use universal precautions when handling human blood and body fluids. Dispose of used reagent vials in accordance with good laboratory practices. Before disposal, waste from possible biohazardous samples should be inactivated using appropriate procedures.

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

Introduction

IT 1-2-3 Sample Purification Kits

For a complete overview of matrices and kits, see the table at the end of this booklet.

BioFire Defense offers additional kits for purification of DNA and RNA:

- **IT 1-2-3 Platinum Path Sample Purification Kit**
for magnetic bead purification of biological, environmental, and food samples
- **IT 1-2-3 FLOW Sample Purification Kits**
for purification of blood, air, water, and food samples
- **IT 1-2-3 SWIPE Sample Purification Kit**
for purification of nasal/pus and surface swabs, lymph node aspirates, live culture and powder samples
- **IT 1-2-3 SCOOP Sample Purification Kit**
for purification of stool and soil samples
- **IT 1-2-3 VIBE Sample Purification Kit**
for purification of sputum samples and RNA from blood, nasal, and throat swab samples
- **IT 1-2-3 DNA Sample Purification Kit**
for purification of surface swab, powder, culture, and water/PBS samples

All kits contain protocols that are performed manually and have been simplified and clearly defined to reduce the risk of operator error. They have been designed to purify samples for analysis with BioFire Defense's R.A.P.I.D.[®] and JBAIDS detection systems.

The IT 1-2-3 SWIPE Kit is an upgraded version of the IT 1-2-3 RAPID DNA Purification Kit.

Purifying DNA or RNA

DNA and RNA are present in bacterial or viral pathogens that can be found in biological, environmental or food samples. Such samples include nasal and environmental swabs, water, blood, etc. There are many inhibitors present in some samples that need to be removed with purification protocols for downstream sample analysis. Inhibitors include cellular debris, chemicals, enzymes and other naturally occurring inhibitors that either degrade DNA or RNA or hinder downstream analysis. DNA and RNA extraction and purification from a complex sample is usually necessary before identification and/or quantification steps can be successfully performed. The protocols in this kit employ the steps described below.

Sample Purification involves four steps:

1. DNA/RNA is **Extracted** from the sample (e.g. cells or spores) through lysis. This is achieved by physical agitation and chemical disruption of the cells with bead-beating or heat (heat is adequate for RNA viruses).
2. DNA/RNA is **Bound** and concentrated on the filter (i.e. **Buffer 1**).
3. The DNA/RNA on the filter is **Washed** to remove inhibitors (i.e. **Buffer 2**).
4. The DNA/RNA is **Eluted** from the filter (i.e. **Buffer 3**).

Other steps are often added to increase total DNA/RNA recovered from various sample matrices. For example, a **protease** step is added to the blood protocol to break down unwanted proteins.

Inhibition Controls

Inhibition controls included in a test ensure that a given sample does not inhibit the downstream analysis and that a negative result is real. The troubleshooting section describes what to do if an inhibition control shows that a sample is inhibited. Contact BFDf to see if inhibition controls are available for your assay.

Sample Purification Procedures

IT 1-2-3 SWIPE Sample Purification Kit for Nasal/Pus Swab, Surface Swab, Lymph Node Aspirates, Powder and Culture Samples

This kit and protocols are designed to purify deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) from bacteria or viruses in the following matrices:

Sample		DNA protocol	RNA protocol
Biological	Nasal/Pus Swab	X	
	Lymph Node Aspirates	X	
	Culture, including blood culture	X	X
Environmental	Surface Swab	X	X
	Powder	X	X
	🔄 Water/PBS	X	X

PBS = phosphate buffered saline

Approximate time to process up to 12 samples is 1-2 hours. Ambient temperature is defined as 18-30°C.

Optimal Protocol Definition

Protocols with this symbol  were optimized and verified for the detection of pathogens at low levels with the R.A.P.I.D. and JBAIDS detection systems.

Alternative Protocol Definition

Alternative protocols or tips given with this symbol  have been tested, but not optimized, to add more flexibility for users. These protocols may not remove all inhibitors and may not achieve desired sensitivity.

Equipment Required

- Microcentrifuge capable of spinning 1.5 mL tubes 7,200-16,000 x g (RCF)
- Disruptor Genie (SI-D237) [or Vortex-Genie 2T (SI-T236) with Turbo Mix attachment] and adapter for 2.0 mL tubes (SI-0562) (Scientific Industries).
- Pipettors (Required range: 5-200 μ L) and tips
- **RNA protocols only:** Heat block for 1.5 mL tubes
- **RNA protocols only:** Freezer (non frost-free, -20°C) (for **Carrier RNA** storage, Carrier RNA purchased separately in RNA Module)

IT 1-2-3 SWIPE Kit Contents

- Sample Swabs for surface, powder and culture sampling (40)
- Swab Snippers (2) and Wipes (40)
- Small Bead Tubes with beads (40)
- Small Spin Filters (40)
- Small Receiver Tubes (120)
- Small Transfer Pipets (for measuring 450 μ L) (120)
- **Buffer 1** (Binding Buffer)
- **Buffer 2** (Wash Buffer)
- **Buffer 3** (Elution Buffer)
- SWIPE Instruction Booklet

Additional Items Needed

- **RNA protocols only: Carrier RNA**, provided in the IT 1-2-3 RNA Module (BFDf Part Number ASAY-ASY-0501)
- Blood culture only: Conical tube (10 mL or larger); PBS or other phosphate buffer (pH range 7.2-7.4), PBS packets: BFDf Part Number ASAY-ASY-0061 (water not included); water

Nasal/Pus Swab Protocol (DNA)

This protocol describes how to purify DNA from a nasal or pus swab sample. (A protocol to process RNA from a nasal swab is included in the IT 1-2-3 VIBE Kit.)

Acceptable sample: Nasal or pus sample on a swab and stored in a dry tube (Copan 168C rayon swab with twisted wire or equivalent is recommended).

Note: Calcium alginate swabs with aluminum shafts should not be used.

Preparation:

1. Remove swab from its tube. With provided Swab Snippers, cut off the end of the swab into a Small Bead Tube approximately $\frac{1}{4}$ inch above the head.
2. Discard the remaining part of the swab.
3. Clean Snippers with alcohol wipe.

Lysis:

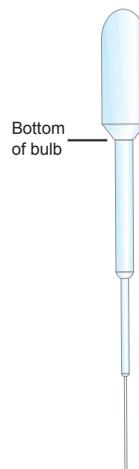
4. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

5. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μ L **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
6. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
7. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
8. Manually transfer Small Spin Filter to a new Receiver Tube (second). Discard old Tube.

Wash filter:

9. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μ L **Buffer 2** (Wash Buffer) to Small Spin Filter.
10. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
11. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
12. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
13. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.



Elute purified sample:

14. Use a Pipettor set to 200 μL to add **Buffer 3** (Elution Buffer) to the spin filter.
15. Incubate for 2 minutes at ambient temperature.
16. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
17. To enhance the recovery of nucleic acids, use the Pipettor set to 200 μL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
18. Incubate for 2 minutes at ambient temperature.
19. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
20. Keep Receiver Tube containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 17-19 (re-elution) may be skipped if optimal sensitivity is not required.
No Pipettor option	Steps 14 and 17 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL Buffer 3 (Elution Buffer)” or to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps). This option can be used (1) if optimal sensitivity is not required or (2) if using Freeze-dried reagents without a Reconstitution Buffer that allows hydration of reagents with sample only.

Lymph Node Aspirate Protocol (DNA) ↑

This protocol describes how to purify DNA from a lymph node aspirate sample.

Acceptable sample: Lymph node aspirate sample (approximately 20 μL) (can be collected on a swab and stored in a dry tube (Copan 168C rayon swab with twisted wire or equivalent is recommended). **Note:** Calcium alginate swabs with aluminum shafts should not be used.) If the sample does not contain liquid and consists of solid tissue larger than the equivalent of 20 μL , the solid tissue should be minced, mashed, or drawn through a needle in order to create a slurry of loose cells that can be picked up with a swab (see step 1 below.)

Preparation:

1. Use a Sample Swab to absorb liquid portion of the sample. If no liquid is present, touch sample with the swab in order to collect as much material as possible from the outside of the solid sample. A small amount of solid sample can be picked up with the swab (only enough to cover the lower quarter of the swab tip, do not pick up a large amount of solid sample or filter will clog).
2. If sample is stored on a swab: With provided Swab Snippers, cut off the end of the swab into a Small Bead Tube approximately $\frac{1}{4}$ inch above the head. Discard the remaining part of the swab. Clean Snippers with alcohol wipe, if used.

Lysis:

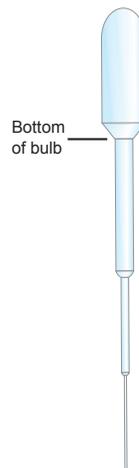
3. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

4. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
5. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
6. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
7. Manually transfer Small Spin Filter to a new Receiver Tube (second). Discard old Tube.

Wash filter:

8. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.



9. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
10. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
11. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
12. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.

Elute purified sample:

13. Use a Pipettor set to 200 µL to add **Buffer 3** (Elution Buffer) to the spin filter.
14. Incubate for 2 minutes at ambient temperature.
15. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
16. To enhance the recovery of nucleic acids, use the Pipettor set to 200 µL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
17. Incubate for 2 minutes at ambient temperature.
18. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
19. Keep Receiver Tube containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter Protocol	Steps 16-18 (re-elution) may be skipped if optimal sensitivity is not required.
No Pipettor Option	Steps 13 and 16 can be replaced with: "Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 µL Buffer 3 (Elution Buffer)" or to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps). This option can be used (1) if optimal sensitivity is not required or (2) if using Freeze-dried reagents without a Reconstitution Buffer that allows hydration of reagents with sample only.

Surface Swab Protocol (DNA) ↑

This protocol is designed to purify DNA from pathogens present on surface swab samples.

Acceptable sample: Sample Swabs are included in the kit for surface swabbing. The swab sampling protocol is listed below. To maximize recovery of pathogens/ nucleic acid from the swab, the swab is broken into the tube for bead beating and purified.

Surface Swab Sampling:

1. Wet a Sample Swab with liquid in a Small Bead Tube.
2. Thoroughly wipe a 2-inch x 2-inch surface area with the Swab.
3. Break the Sample Swab off in the Bead Tube at the swab breakpoint.

Lysis:

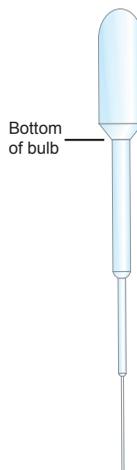
4. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

5. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
6. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
7. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
8. Manually transfer Small Spin Filter to a new Receiver Tube (second) by hand. Discard old Tube.

Wash filter:

9. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
10. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
11. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
12. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
13. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.



Elute purified sample:

14. Use a Pipettor set to 200 µL to add **Buffer 3** (Elution Buffer) to the spin filter.
15. Incubate for 2 minutes at ambient temperature.
16. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
17. To enhance the recovery of nucleic acids, use the Pipettor set to 200 µL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
18. Incubate for 2 minutes at ambient temperature.
19. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
20. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 17-19 (re-elution) may be skipped if optimal sensitivity is not required.
No Pipettor option	Steps 14 and 17 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 µL Buffer 3 (Elution Buffer)” and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps). This option can be used if (1) optimal sensitivity is not required or (2) if using Freeze-dried reagents without a Reconstitution Buffer that allows hydration of reagents with sample only.
Other swabs	If surface swabs are taken with a swab other than a Sample Swab, cut the head of the swab into the Small Bead Tube with the Swab Snippers. (See Nasal swab protocol.)
Direct testing: Bead beat only	Some surface swab samples do not contain downstream inhibitors and can be tested directly after bead beating. In this case, follow the protocol through step 4 and proceed to downstream analysis. Inhibition controls can be used with this protocol.

Surface Swab Protocol (RNA) ↑

This protocol is designed to purify RNA from pathogens present on surface swab samples. **Acceptable sample:** Sample Swabs are included in the kit for surface swabbing. The swab sampling protocol is listed below. To maximize recovery of pathogens/nucleic acid from the swab, the swab is broken into the tube for bead beating and purified.

Surface Swab Sampling:

1. Wet a Sample Swab with liquid in a Small Bead Tube.
2. Thoroughly wipe a 2-inch x 2-inch surface area with the Swab.
3. Break the Sample Swab off in the Bead Tube at the swab breakpoint.

Preparation:

4. Add 16 μL of **Carrier RNA** to each Small Bead Tube containing a swab. Cap Tube.

Lysis:

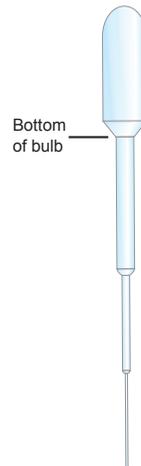
5. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

6. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
7. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
8. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
9. Manually transfer Small Spin Filter to a new Receiver Tube (second) by hand. Discard old Tube.

Wash filter:

10. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
11. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
12. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
13. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.



14. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.
15. To remove more residual ethanol (important for RNA), incubate the uncapped tube with the Small Spin Filter inside, for 5 minutes in a dry heat block set to 88-95°C.

Elute purified sample:

16. Use a Pipettor set to 200 µL to add **Buffer 3** (Elution Buffer) to the spin filter.
17. Incubate for 2 minutes at ambient temperature.
18. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
19. To enhance the recovery of nucleic acids, use the Pipettor set to 200 µL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
20. Incubate for 2 minutes at ambient temperature.
21. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
22. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 19-21 (re-elution) may be skipped if optimal sensitivity is not required.
Reduce Pipettor option	Steps 16 and 19 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 µL Buffer 3 (Elution Buffer) and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps), if optimal sensitivity is not required.
Other swabs	If surface swabs are taken with a swab other than a Sample Swab, cut the head of the swab into the Small Bead Tube with the Swab Snippers. (See Nasal swab protocol.)
DNA and RNA from same sample	The RNA protocol given here for surface swab samples can be used to purify both DNA and RNA from the same sample. Optimal sensitivity is not guaranteed for DNA detection using this RNA protocol.

Powder Protocol (DNA)

This protocol is designed to purify DNA from pathogens present in powder samples.

Acceptable sample: A small amount (~ 1 mg) of powder picked up with a Sample Swab (see sampling protocol below).

Powder Swab Sampling:

1. Wet a Sample Swab with liquid in a Small Bead Tube.
2. Touch the powder with only the very tip of the moist Swab. (**Note:** Large quantities of powder can clog the filter in succeeding steps).
3. Break the Sample Swab off in the Bead Tube at the swab breakpoint.

Lysis:

4. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

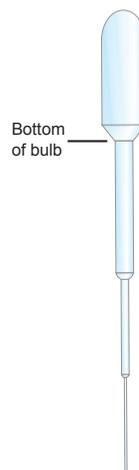
5. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
6. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
7. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
8. Manually transfer Small Spin Filter to a new Receiver Tube (second). Discard old Tube.

Wash filter:

9. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
10. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
11. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
12. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
13. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.

Elute purified sample:

14. Use a Pipettor set to 200 μL to add **Buffer 3** (Elution Buffer)



to the spin filter.

15. Incubate for 2 minutes at ambient temperature.
16. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
17. To enhance the recovery of nucleic acids, use the Pipettor set to 200 μ L and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
18. Incubate for 2 minutes at ambient temperature.
19. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
20. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 17-19 (re-elution) may be skipped if optimal sensitivity is not required.
No Pipettor option	Steps 14 and 17 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μ L Buffer 3 (Elution Buffer)” and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps). This option can be used if (1) optimal sensitivity is not required or (2) if using Freeze-dried reagents without a Reconstitution Buffer that allows hydration of reagents with sample only.
Direct testing: Bead beat only	Some powder samples do not contain downstream inhibitors and can be tested directly after bead beating. In this case, follow the protocol through step 4 and proceed to downstream analysis. With powder samples (usually highly concentrated) it is advisable to prepare a ten-fold dilution of the bead-beaten sample in a Small Receiver Tube with Buffer 3 (for example 40 μ L sample plus 360 μ L Buffer 3). Inhibition controls can be used with this protocol.

Powder Protocol (RNA)

This protocol is designed to purify RNA from pathogens present in powder samples.

Acceptable sample: A small amount (~ 1 mg) of powder picked up with an Sample Swab (see sampling protocol below).

Powder Swab Sampling:

1. Wet a Sample Swab with liquid in a Small Bead Tube.
2. Touch the powder with only the very tip of the moist Swab. (**Note:** Large quantities of powder can clog the filter in proceeding steps).
3. Break the Sample Swab off in the Bead Tube at the swab breakpoint.

Preparation:

4. Add 16 μL of **Carrier RNA** to each Small Bead Tube containing a swab. Cap Tube.

Lysis:

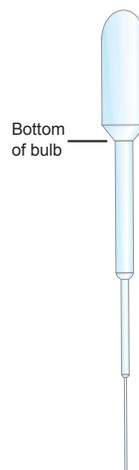
5. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

6. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
7. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
8. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
9. Manually transfer Small Spin Filter to a new Receiver Tube (second) by hand. Discard old Tube.

Wash filter:

10. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
11. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
12. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
13. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
14. Manually transfer Small Spin Filter to a new Receiver Tube



(fourth). Discard old Tube.

15. To remove more residual ethanol (important for RNA), incubate the uncapped tube with the Small Spin Filter inside, for 5 minutes in a dry heat block set to 88-95°C.

Elute purified sample:

16. Use a Pipettor set to 200 μL to add **Buffer 3** (Elution Buffer) to the spin filter.
17. Incubate for 2 minutes at ambient temperature.
18. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
19. To enhance the recovery of nucleic acids, use the Pipettor set to 200 μL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
20. Incubate for 2 minutes at ambient temperature.
21. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
22. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 19-21 (re-elution) may be skipped if optimal sensitivity is not required.
Reduce Pipettor option	Steps 16 and 19 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL Buffer 3 (Elution Buffer)” and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps), if optimal sensitivity is not required.
DNA and RNA from same sample	The RNA protocol given here for powder samples can be used to purify both DNA and RNA from the same sample. Optimal sensitivity is not guaranteed for DNA detection using this RNA protocol.

Culture Protocol (Including Blood Culture; DNA) ↑

Use the alternative optimized protocol for processing plated colonies intended for use with *in vitro* diagnostic products.

Organisms cultured on solid (plates) or in liquid media generally produce highly concentrated samples. Sample volumes for this protocol are therefore small.

Acceptable sample: Bacterial cultures or viral cell culture with appropriate culture media.

1. **For plated colonies or plaques:** touch a Sample Swab to the colony or plaque and break off the swab at the breakpoint into a Small Bead Tube.

For liquid culture: dip a Sample Swab in culture media, allow it to soak for 3-5 seconds and break off the swab at the breakpoint in a Small Bead Tube. (Alternatively, pipette 40-50 μL the liquid culture directly into a bead tube.)

For blood culture: First dilute the blood culture media by adding 50 μL of blood culture media to 5 mL of PBS in a conical tube (or equivalent). Mix well. Transfer 50 μL of the diluted blood culture media into a Small Bead Tube.

Lysis:

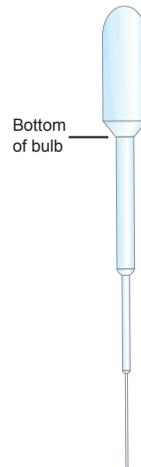
2. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

3. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
4. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
5. Centrifuge for 2 minutes at maximum speed (min. 7,200 *g*).
6. Manually transfer Small Spin Filter to a new Receiver Tube (second). Discard old Tube.

Wash filter:

7. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
8. Centrifuge for 2 minutes at maximum speed (min. 7,200 *g*).
9. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
10. Centrifuge for 3 minutes at maximum speed (min. 7,200 *g*) to remove residual ethanol.



- Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.

Elute purified sample:

- Use a Pipettor set to 200 μL to add **Buffer 3** (Elution Buffer) to the spin filter.
- Incubate for 2 minutes at ambient temperature.
- Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
- To enhance the recovery of nucleic acids, use the Pipettor set to 200 μL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
- Incubate for 2 minutes at ambient temperature.
- Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
- Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

 Alternative Optimized Protocol	
Direct testing: Bead beat only	Some bacterial colonies can be tested directly after bead beating. Follow the protocol through step 2 and immediately prior to testing: 1) Add 5 μL bead-beaten sample to a Small Receiver Tube 2) Add 500 μL Buffer 3 and proceed with downstream analysis Use of inhibition controls with this protocol is recommended.
	
Shorter protocol	Steps 15-17 (re-elution) may be skipped if optimal sensitivity is not required.
No Pipettor option	Steps 12 and 15 can be replaced with: "Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL Buffer 3 (Elution Buffer)" and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps). This option can be used if (1) optimal sensitivity is not required or (2) if using Freeze-dried reagents without a Reconstitution Buffer that allows hydration of reagents with sample only.

Culture Protocol (RNA)

Organisms cultured on solid (plates) or in liquid media generally produce highly concentrated samples. Sample volumes for this protocol are therefore small.

Acceptable sample: Viral cell culture with appropriate culture media.

1. **For plated colonies or plaques:** touch a Sample Swab to the plaque and break off the swab at the breakpoint into a Small Bead Tube.

For liquid culture: dip a Sample Swab in culture media, allow it to soak for 5 seconds and break off the swab at the breakpoint in a Small Bead Tube. (Alternatively, pipette 40-50 μ L the liquid culture directly into a bead tube.)

Preparation:

2. Add 16 μ L of **Carrier RNA** to each Small Bead Tube containing a swab. Cap Tube.

Lysis:

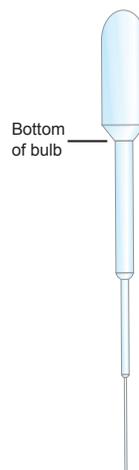
3. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

4. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μ L **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
5. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
6. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
7. Manually transfer Small Spin Filter to a new Receiver Tube (second) by hand. Discard old Tube.

Wash filter:

8. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μ L **Buffer 2** (Wash Buffer) to Small Spin Filter.
9. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
10. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
11. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
12. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.



13. To remove more residual ethanol (important for RNA), incubate the uncapped tube with the Small Spin Filter inside, for 5 minutes in a dry heat block set to 88-95°C.

Elute purified sample:

14. Use a Pipettor set to 200 µL to add **Buffer 3** (Elution Buffer) to the spin filter.
15. Incubate for 2 minutes at ambient temperature.
16. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
17. To enhance the recovery of nucleic acids, use the Pipettor set to 200 µL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
18. Incubate for 2 minutes at ambient temperature.
19. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
20. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 17-19 (re-elution) may be skipped if optimal sensitivity is not required.
Reduce Pipettor option	Steps 14 and 17 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 µL Buffer 3 (Elution Buffer)” and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps), if optimal sensitivity is not required.

Water/PBS Protocol (DNA) Alternative Protocol

This protocol is an alternative to the Water and PBS protocols in the IT 1-2-3 FLOW Kit. It processes less liquid, therefore sensitivity of detection is not as good with this protocol, but it is a good option for users with only small equipment (smaller centrifuge, etc.). This protocol has not been used for sensitivity verification with the RAPID or JBAIDS detection systems.

Acceptable sample: Water or PBS (with or without Triton X-100).

1. Pipette 100 μL Water or PBS into a Small Bead Tube (alternatively, for 40 μL , dip a Sample Swab into the liquid, allow it to soak for 3-5 seconds and break off the swab at the breakpoint into a Small Bead Tube).

Lysis:

2. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

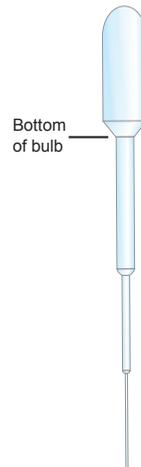
3. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
4. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
5. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
6. Manually transfer Small Spin Filter to a new Receiver Tube (second). Discard old Tube.

Wash filter:

7. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
8. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
9. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
10. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.
11. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.

Elute purified sample:

12. Use a Pipettor set to 200 μL to add **Buffer 3** (Elution Buffer) to the spin filter.



13. Incubate for 2 minutes at ambient temperature.
14. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
15. To enhance the recovery of nucleic acids, use the Pipettor set to 200 μ L and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
16. Incubate for 2 minutes at ambient temperature.
17. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
18. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 15-17 (re-elution) may be skipped if optimal sensitivity is not required.
No Pipettor option	<p>Steps 12 and 15 can be replaced with:</p> <p>“Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL Buffer 3 (Elution Buffer)” and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps).</p> <p>This option can be used if (1) optimal sensitivity is not required or (2) if using Freeze-dried reagents without a Reconstitution Buffer that allows hydration of reagents with sample only.</p>
Direct testing: Bead beat only	Some samples do not contain downstream inhibitors and can be tested directly after bead beating. In this case, follow the protocol through step 2 and proceed to downstream analysis. To dilute out inhibitors, prepare a ten-fold dilution of the bead-beaten sample in a Small Receiver Tube with Buffer 3 (for example 40 μ L sample plus 360 μ L Buffer 3). Inhibition controls can be used with this protocol.

Water/PBS Protocol (RNA) Alternative Protocol

This protocol is an alternative to the Water and PBS protocols in the IT 1-2-3 FLOW Kit. It processes less liquid, therefore sensitivity of detection is not as good with this protocol, but it is a good option for users with only small equipment (smaller centrifuge, etc.). This protocol has not been used for sensitivity verification with the RAPID or JBAIDS detection systems.

Acceptable sample: Water or PBS (with or without Triton X-100).

1. Pipette 100 μL Water or PBS into a Small Bead Tube (alternatively, for 40 μL , dip a Sample Swab into the liquid, allow it to soak for 3-5 seconds and break off the swab at the breakpoint into a Small Bead Tube).

Preparation:

2. Add 16 μL of **Carrier RNA** to each Small Bead Tube containing a swab. Cap Tube.

Lysis:

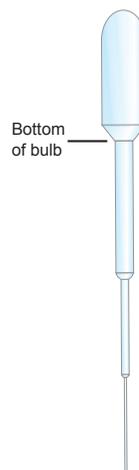
3. Place Bead Tubes into 2 mL tube holder on the Disruptor Genie (or Vortex Genie with Turbo Mix) and Bead Beat on the highest setting for 5 minutes with lid down to disrupt cells or viruses and release nucleic acids.

Bind filter:

4. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 1** (Binding Buffer) to Small Bead Tube containing bead beaten sample. Keep transfer pipet for next step.
5. Use same transfer pipet to transfer liquid from Small Bead Tube to Small Spin Filter (packaged in a Small Receiver Tube), avoiding beads in the Bead Tube.
6. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
7. Manually transfer Small Spin Filter to a new Receiver Tube (second) by hand. Discard old Tube.

Wash filter:

8. Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 μL **Buffer 2** (Wash Buffer) to Small Spin Filter.
9. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
10. Manually transfer Small Spin Filter to a new Receiver Tube (third). Discard old Tube.
11. Centrifuge for 3 minutes at maximum speed (min. 7,200 g) to remove residual ethanol.



12. Manually transfer Small Spin Filter to a new Receiver Tube (fourth). Discard old Tube.
13. To remove more residual ethanol (important for RNA), incubate the uncapped tube with the Small Spin Filter inside, for 5 minutes in a dry heat block set to 88-95°C.

Elute purified sample:

14. Use a Pipettor set to 200 µL to add **Buffer 3** (Elution Buffer) to the spin filter.
15. Incubate for 2 minutes at ambient temperature.
16. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
17. To enhance the recovery of nucleic acids, use the Pipettor set to 200 µL and repeat elution by pipetting the eluate (spin-through) from the bottom of the Receiver Tube back onto the Small Spin Filter.
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19. Centrifuge for 2 minutes at maximum speed (min. 7,200 g).
20. Keep Receiver Tube (fourth) containing the purified sample and test as soon as possible.

Storage and downstream analysis:

If the purified sample will not be tested within 30 minutes, it is recommended to store it at 2-8°C and using it for downstream analysis within 4 hours. If testing does not occur within 4-8 hours, sensitivity may be affected due to degradation of target template.

	
Shorter protocol	Steps 17-19 (re-elution) may be skipped if optimal sensitivity is not required.
Reduce Pipettor option	Steps 14 and 17 can be replaced with: “Use a Small Transfer Pipet (fill to bottom of bulb) to add 450 µL Buffer 3 (Elution Buffer)” and to repeat elution (if re-elution is necessary, the same transfer pipet must be used for both steps) if optimal sensitivity is not required.
DNA and RNA from same sample	The RNA protocol given here for water/PBS samples can be used to purify both DNA and RNA from the same sample. Optimal sensitivity is not guaranteed for DNA detection using this RNA protocol.

Troubleshooting

Symptom	Resolution
Spin Filter Clogging	If the Small Spin Filter clogs, follow protocol up to the addition of Buffer 2 . Before adding Buffer 2 , pour liquid off of the top of the filter and continue with the protocol.
Inhibited Sample	<p>Sometimes unknown inhibitors of down stream analysis are not adequately removed from a purified sample. If amplification does not occur as expected or an inhibition control shows inhibitors are present, perform a ten-fold dilution of that sample in Reagent Grade Water or Buffer 3. A ten-fold dilution is usually adequate to remove the effects of the inhibitors, however sensitivity is decreased.</p> <p>Note: If inhibition controls are used, refer to the reagent product insert for specific directions.</p>

Ordering Information

Sample Purification Kits and Supplies

Item	Contents	Part No.
IT 1-2-3 DNA Sample Purification Kit	Sample Purification and Extraction Kit for minimally trained technicians to extract DNA from environmental sources	3800
IT 1-2-3 SWIPE Sample Purification Kit*	Sample Purification Kit for purification of nasal swab, surface swab, live culture and powder samples	ASAY-ASY-0005
IT 1-2-3 FLOW Sample Purification Kit**	Sample Purification Kit for purification of blood, air, water, food and body fluid samples	ASAY-ASY-0004
IT 1-2-3 VIBE Sample Purification Kit	Sample Purification Kit for purification of sputum samples and RNA from blood and nasal swab samples	ASAY-ASY-0500
IT 1-2-3 SCOOP Sample Purification Kit	Sample Purification Kit for purification of stool and soil samples	ASAY-ASY-0502
IT 1-2-3 QFLOW DNA Sample Purification Kit*	Sample Purification Kit for purification of blood, air, water, food and body fluid samples	ASAY-ASY-0503
IT 1-2-3 QFLOW RNA Sample Purification Kit	Sample Purification Kit for purification of blood, air, water, food and body fluid samples	ASAY-ASY-0504
IT 1-2-3 Platinum Path Sample Purification Kit	Sample Purification Kit for magnetic bead purification of biological, environmental, and food samples	ASAY-ASY-0120
IT 1-2-3 RNA Module	An accessory to the SWIPE, FLOW, VIBE, and QFLOW ^{DNA} purification kits and contains items for the purification of 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

*These purification kits require the RNA Module (ASAY-ASY-0501) for some preparation applications.

†Large bead tube adapter (P/N PREP-ASY-0001) is required for this kit, but not included.

Sample Type		FLOW	SWIPE	VIBE	SCOOP	Platinum Path	RNA Module
Biological	Whole Blood	DNA X				X	
		RNA		X		X	X
	Nasal/Pus Swabs	DNA	X			X	
		RNA		X		X	X
	Culture	DNA	X			X	
		RNA		X		X	X
	Sputum	DNA		X		X	X
		RNA			X	X	X
	Stool	DNA	X		X	X	
	Gastric Wash	DNA				X	
	Lymph Node Aspirates	DNA		X		X	
	Cerebral Spinal Fluid	DNA				X	
	Cerebral Spinal Fluid	RNA				X	
	Nasopharyngeal Swab and Throat Swab	RNA				X	
Environmental	Air (PBS)	DNA X	☞			X	
		RNA X	☞			X	X
	Surface Swabs	DNA	X			X	
		RNA		X		X	X
	Powder	DNA	X			X	
		RNA		X		X	X
	Water	DNA X	☞			X	
	Soil	DNA			X	X	
	Milk	DNA	X			X	
	Mixed Greens	DNA	X			X	
Food	Ground Beef	DNA	X			X	
	Tuna Salad	DNA	X			X	

☞ = Alternative protocols



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our Customer Service Department
at BioFire Defense.*

