Kit Part No: ASAY-ASY-0502

IT 1-2-3™ SCOOP

Sample Purification Kit

Instruction Booklet

Manufactured by
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IT 1-2-3™ SCOOP Sample Purification Instruction Booklet
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Table of Contents

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
g	Gravity (= RCF)
LOD	Limit of Detection
mL	Milliliter
MSDS	Material Safety Data Sheet
PBS	Phosphate-buffered saline
RCF	Relative Centrifugal Force (= g)
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 MSDS 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 http://biofiredefense.com/sample-purification

BioFire Defense offers these other kits[1] 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 QFLOW Sample Purification Kits**
  for purification of blood, serum, air, water, 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's Ruggedized Advanced Pathogen Identification Device (R.A.P.I.D.®) and Joint Biological Agent Identification and Diagnostic System (JBAIDS) detection systems.

[1]These kits are controlled and require an export license.

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**).
4. The DNA/RNA on the filter is **Washed** to remove inhibitors (i.e. **Buffer 2**).
5. 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 SCOOP Sample Purification Kit for Stool and Soil

This kit is designed to process 0.5 g Soil, or 0.5 mL Stool samples resuspended 1:10 in Cary Blair medium.

Sample		DNA protocol
Biological	Stool	**X**
Environmental	Soil	**X**

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

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 plus adapter for 2.0 mL tubes (SI-0562) (Scientific Industries).
- Pipettors (Required range: 100-1000 µL) and tips
- **Soil samples only:** Scale is recommended

Contents in the IT 1-2-3 SCOOP Kit

- SCOOP Bead Tubes (40)
- SCOOP Spin Filters (40)
- SCOOP Receiver Tubes (80)
- Small Transfer Pipets (for measuring 450 µL) (80)
- Sample Transfer Pipets (3 mL capacity) (40)
- Mix Tubes
- **Buffer SCOOP 1A**
- **Buffer SCOOP 1B**
- **Protein Precipitation Solution**
- **Binding Suspension**
- **Buffer 2** (Wash Buffer)
- **Buffer 3** (Elution Buffer)
- SCOOP Instruction Booklet

Additional Items needed for Stool Protocol

- Cary Blair Stool Collection Vials: BFDF Part Number ASAY-ASY-0063 (Fisher Protocol Cary Blair Medium Vials (#23-005-47), or equivalent)
- Stool Concentration Filters: BFDF Part Number ASAY-ASY-0064 (Meridian Bioscience, Inc. PARA-PAK Macro-CON (#970120, Fisher Cat No. 14-910-147), or equivalent)

Soil Protocol (DNA)

This protocol is designed to purify DNA from pathogens present in soil. Note that some soil types contain high levels of inhibitors that are not entirely removed with this protocol, which was optimized for a mixture of topsoil (loam), sand and clay. Soils with higher organic content have more inhibitors and the desired sensitivity may not be achieved with such soils. See note at end of protocol for how to deal with inhibitors in the purified sample.
Acceptable sample: 0.5 g soil.

Preparation:

1. Add 0.5 g (± 0.1 g) soil to SCOOP Bead Tube (0.5 g soil is approximately equivalent to 0.5 mL, fill to the 1.0 mL mark on the bead tube).
2. Use Pipettor to add 980 µL of **Buffer SCOOP 1A** to the tube containing the soil.
3. Use Pipettor to add 120 µL **Buffer SCOOP 1B** to the same tube. Cap tube.

Lysis:

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

Protein Precipitation:

5. Centrifuge for 2 minutes at maximum speed (minimum 7,200 *g*).
6. Use a Small Transfer Pipet to transfer liquid to a SCOOP Receiver Tube taking care to avoid beads.
7. Use Pipettor to add 250 µL **Protein Precipitation Solution** to receiver tube containing liquid. Cap tube.
8. Mix tube contents by inverting 10 times.
9. Centrifuge for 5 minutes at maximum speed (minimum 7,200 *g*).

Bind and place on filter:

10. Use a Small transfer pipet to transfer supernatant to a Mix Tube.
11. Use Pipettor to add 1000 µL (1 mL) of well-mixed **Binding Suspension** to Mix tube containing supernatant. (Vortex Binding Suspension extensively to get the solid at the bottom completely into suspension.) Cap tube.
12. Mix tube by inverting it for 2 minutes by hand.
13. Filter Load #1: Use a Sample transfer pipet (the larger 3 mL pipet) to load 0.6 mL of the well-mixed suspension onto a SCOOP Spin Filter**.** Keep the transfer pipet in the Mix Tube until Spin Filter is fully loaded.

14. Centrifuge for **2** minutes at maximum speed (minimum 7,200 *g*).
15. Pour off flow-through into waste container.
16. Repeat steps 13-15 three more times until suspension is fully loaded onto the Spin Filter for Filter loads #2, #3 and #4. **Note:** Ensure the sample gets loaded into the correct Spin Filter during each load.

Wash filter:

17. Use Pipettor to add 500 µL **Buffer 2** to Spin Filter and gently stir the matrix with the pipette tip, taking care not to puncture the white membrane. Cap tube.
18. Centrifuge for 2 minutes at maximum speed (minimum 7,200 *g*).
19. Pour off flow-through into waste container.

Dry spin:

20. Centrifuge for 3 minutes at maximum speed (minimum 7,200 *g*).
21. Move Spin Filter to a clean SCOOP Receiver Tube.
22. Incubate at ambient temperature with caps open for 5 minutes to dry the matrix.

Elute purified sample:

23. Use Pipettor to add 200 µL **Buffer 3** to the Spin Filter and gently stir the matrix with the pipette tip, taking care not to puncture the white membrane.
24. Centrifuge for 2 minutes at maximum speed (minimum 7,200 *g*).
25. 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 2-8ºC 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.

Note on soil sample inhibitors

If amplification does not occur or inhibition control shows inhibitors are present, add less purified sample per reaction: 2 µL per Freeze-dried reagent tube (1 µL per reaction) plus 18 µL water and 20 µL Reconstitution Buffer. The addition of less purified sample will dilute inhibitors, but decrease sensitivity due to fewer target molecules per reaction.

Stool Protocol (DNA)

This protocol is designed to purify DNA from pathogens present in stool.
Acceptable sample: 1.5 g Solid stool or at least 0.5 mL stool in Cary Blair Transport Media.

Follow these pre-treatment steps for stool:

A. Add 1.5 g stool using the scoop provided in a Cary Blair transport media tube. (A heaping scoop is 1.5 g. If sample is liquid, add 1.5 mL.)

B. Add stool to a Cary Blair transport media tube (containing 15 mL media) and briefly shake or vortex to mix well.

C. Allow stool to equilibrate in Cary Blair for approximately 30 minutes.

D. Filter sample using a stool concentrator filter tube (0.6 mm pore size) by placing in Cary Blair tube, flipping upside down, and tapping tube on a hard surface so the stool mixture flows through the filter.

Preparation:

1. Add 450 µL diluted/filtered stool to SCOOP Bead Tube with Pipettor set to 450 µL.
2. Use Pipettor to add 980 µL of **Buffer SCOOP 1A** to the tube containing the stool.
3. Use Pipettor to add 120 µL **Buffer SCOOP 1B** to the same tube. Cap tube.

Lysis:

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

Protein Precipitation:

5. Centrifuge for 2 minutes at maximum speed (minimum 7,200 *g*).
6. Using a Small Transfer Pipet to transfer liquid to a SCOOP Receiver Tube, taking care to avoid beads.
7. Use Pipettor to add 250 µL **Protein Precipitation Solution** to receiver tube containing liquid. Cap tube.
8. Mix tube contents by inverting 10 times.
9. Centrifuge for 5 minutes at maximum speed (minimum 7,200 *g*).

Bind and place on filter:

10. Use a Small transfer pipet to transfer supernatant to a Mix Tube.
11. Use Pipettor to add 1000 µL (1 mL) of well-mixed **Binding Suspension** to Mix

tube containing supernatant. (Vortex Binding Suspension extensively to get the solid at the bottom completely into suspension.) Cap tube.

12. Mix tube by inverting it for 2 minutes by hand.
13. Filter Load #1: Use a Sample transfer pipet (the larger 3 mL pipet) to load 0.6 mL of the well-mixed suspension onto a SCOOP Spin Filter. Keep the transfer pipet in the Mix Tube until Spin Filter is fully loaded.
14. Centrifuge for **2** minutes at maximum speed (minimum 7,200 *g*).
15. Pour off flow-through into waste container.
16. Repeat steps 13-15 three more times until suspension is fully loaded onto the Spin Filter for Filter loads #2, #3 and #4. **Note:** Ensure the sample gets loaded into the correct Spin Filter during each load.

Wash filter:

17. Use Pipettor to add 500 µL **Buffer 2** to Spin Filter and gently stir the suspension with the pipette tip, taking care not to puncture the white membrane. Cap tube.
18. Centrifuge for 2 minutes at maximum speed (minimum 7,200 *g*).
19. Pour off flow-through into waste container.

Dry spin:

20. Centrifuge for 3 minutes at maximum speed (minimum 7,200 *g*).
21. Move Spin Filter to a clean SCOOP Receiver Tube.
22. Incubate at ambient temperature with caps open for 5 minutes to dry the matrix.

Elute purified sample:

23. Use Pipettor to add 200 µL **Buffer 3** to the Spin Filter and gently stir the suspension with the pipette tip, taking care not to puncture the white membrane.
24. Centrifuge for 2 minutes at maximum speed (minimum 7,200 *g*).
25. 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 2-8ºC 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.

Troubleshooting

Symptom	Resolution
Spin Filter Clogging	If the SCOOP Spin Filter clogs, gently stir the suspension with a pipette tip, taking care not to puncture the white membrane and spin again.
Inhibited Sample	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. Note: If inhibition controls are used, refer to the reagent product insert for specific directions.
Punctured Membrane	If the SCOOP Spin Filter is punctured, the Binding Suspension and liquid will be spun through into the receiver tube. Remove filter, resuspend beads by pipetting up and down and transfer beads and liquid into a new SCOOP Spin Filter. Continue with protocol from when the puncture occurred.

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	QFLOW DNA	QFLOW RNA	Platinum Path	RNA Module
Biological	Whole Blood	DNA	X				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	
	Gastric Wash	DNA	X				X		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		X	
		RNA	X	➔				X	X	X
	Surface Swabs	DNA		X					X	
		RNA		X					X	X
	Powder	DNA		X					X	
		RNA		X					X	X
	Water	DNA	X	➔			X		X	
	Soil	DNA				X	X		X	
Food	Milk	DNA	X						X	
	Mixed Greens	DNA	X				X		X	
	Ground Beef	DNA	X				X		X	
	Tuna Salad	DNA	X				X		X	

➔ = Alternative protocols

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For additional information regarding our products and applications, please contact our Customer Service Department at BioFire Defense.

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