Kit Part No: ASAY-ASY-0120

IT *1-2-3*™

Platinum Path

Sample Purification Kit

MagBead Kit for Biological, Environmental, and Food Samples

Instruction Booklet

BIOFIRE DEFENSE

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Contents

Abbreviations and Acronyms

BMBL	Biosafety in Microbiological and Biomedical Laboratories
C	Celsius
CSF	Cerebral Spinal Fluid
DFU	Dry Filter Unit
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid
g	Gram
g	Units of Gravity
BFDf	BioFire Defense
JBAIDS	Joint Biological Agent Identification and Diagnostic System
LOD	Limit of Detection
mg	Milligram
mL	Milliliter
MSDS	Material Safety Data Sheet
NPS	Nasopharyngeal Swab
NPW	Nasopharyngeal Wash
PBS	Phosphate-Buffered Saline
PP	Protein Precipitation
R.A.P.I.D.	Ruggedized Advanced Pathogen Identification Device
RNA	Ribonucleic Acid
µL	Microliter (0.000001 liters)
VTM	Viral transport media

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 material safety data sheet (MSDS) provided by BioFire Defense (BFDf).

Precautions

For general biosafety guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories* (5th Edition, February 2007, Centers for Disease Control and Prevention and National Institutes of Health; available online at http://www.cdc.gov/biosafety/publications/bmbl5/).

Handling of Biohazard Wastes

Use universal precautions when handling human blood and body fluids or any sample with potential pathogens. 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 *Guidelines for Protecting the Safety and Health of Health Care Workers,* section 6, Hazardous Waste Disposal (September 1988; National Institute of Occupational Safety and Health (NIOSH) Publication No. 88-119, available online at http://www.cdc.gov/niosh/).

Introduction

IT 1-2-3 Sample Purification Kits

BioFire Defense offers these sample purification kits:

- **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.

IT 1-2-3 Platinum Path Sample Purification Kit for Biological, Environmental, and Food Samples

The Platinum Path Sample Purification Kit is a comprehensive purification kit that can be used to purify a wide array of biological, environmental, and food samples. The protocols use magnetic beads to isolate the DNA or RNA—a technique that has proven to be simple and very efficient.

Purifying DNA or RNA

DNA and RNA are present in bacterial or viral pathogens that can be found in biological, environmental or food samples. There are many inhibitors present in most samples that need to be removed with purification protocols. 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 main steps:

1. DNA/RNA is **Extracted** from the sample (e.g., cells, spores, or viruses) through lysis. This is achieved by physical agitation and chemical disruption of the cells with bead-beating.
2. DNA/RNA is **Bound** to magnetic beads (well 1 in MagBead strip tube).
3. The DNA/RNA on the magnetic beads is **Washed** to remove inhibitors (wells 2–4 in MagBead strip tube).
4. The DNA/RNA is **Eluted** from the magnetic beads (well 5 in MagBead strip tube).

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

Materials

Equipment Required

- Disruptor Genie™ (SI-D237) [or Vortex-Genie® 2T (SI-T236) with Turbomix™ attachment] and adapter for 2.0 mL tubes (SI-0562) (Scientific Industries).
- Pipettors (Required range: 20–800 µL) and tips
- Refrigerator, 2–8°C
- Peg racks (1 rack per 8 samples; VWR 82024-496)
- BioControl PickPen® 1-M Magnetic Wand[1] (23001, Sunrise Science)
- BioControl PickPen Tip box[1], reusable, holds 96 tips (34196, Sunrise Science)
- Microcentrifuge capable of 2,000 x *g* (for sputum, stool, or soil only)

IT 1-2-3 Platinum Path Kit Contents (40 extractions)

- Sample Swabs (1 bag)
- Swab Snippers and Wipes (1 bag)
- Medium Bead Tubes with beads (40)
- Small Receiver Tubes (120)
- Small Transfer Pipets (2 bags of 20)
- MB Binding Buffer (bottle)
- SCOOP Lysis Buffer (bottle)
- SCOOP PP Buffer 1 (bottle)[2]
- SCOOP PP Buffer 2 (bottle)[2]
- MagBead strip tubes (40)
- Waste covers for strip tubes (40)
- Antifoam powder (1)
- Water (bottle)
- Protocol Instruction Booklet (1)
- Protease Module (store at 4°C)
  - FLOW protease (bottle)
  - VIBE protease (tube)
- Colored dots for sample tracking

Additional Items Needed

- Biocontrol PickPen Tips[1] (Sunrise Science)
- Food Samples Only: Filtered blender bags (ASAY-ASY-0060), PBS packets (ASAY-ASY-0061), scale (optional), 50 mL conical tube, and water.
- Air Samples Only (DFU): PBS packets (ASAY-ASY-0061), Triton® X-100 (ASAY-ASY-0062), and water.
- Stool Only: Cary-Blair Transport Media (ASAY-ASY-0063), and stool concentrators (ASAY-ASY-0064).
- For routine serum testing: Additional VIBE Protease (ASAY-SUB-0509)
- For sputum pre-treatment: Snap n' Digest (Scientific Device Laboratory)

1. Product discontinued. Contact distributor for replacement magnetic tools and accessories.
2. Components of this product are licensed from MO BIO under certain patents owned or controlled by MO BIO for use in the Platinum Path Kit. No other rights, express or implied, are conveyed by the sale of this product.

Sample Purification Procedures

These protocols are designed to purify deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) from bacteria or viruses in the following matrices. The main protocol starts on the next page. Specific sample directions are given on the page numbers specified:

Sample		DNA protocol on these pages	RNA protocol* on these pages
Biological	Whole blood	8	9
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	Gastric wash	10	
	Culture, including blood culture	10	10
	Direct Testing of Bacterial Colonies	11	
	Cerebral spinal fluid (CSF)	11-12	12
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	Pus swab	14-15	
	Lymph node aspirates	14-15	
	Nasal swab	15	15
	Nasopharyngeal swab (NPS) and Nasopharyngeal wash (NPW)		15-16
	Nasopharyngeal swab (NPS) and throat swab		16
	Stool	16-17	
Food	Mixed greens	18	
	Milk	18-19	
	Ground beef	18	
	Tuna salad	19	
Environmental	Water	19	
	Air samples in PBS	20	20
	Powder	20-21	21
	Surface swab	21-22	21-22
	Soil	22-23	

*DNA protocols may purify RNA from all sample matrices listed, but RNA protocols have been validated only for those indicated.

Approximate time to process 1–12 samples is 20–60 min.
Room temperature is defined as 18–30 ºC.

Main Protocol

Preparation

1. **Add powdered antifoam to medium bead tube** by scraping a small amount of antifoam from the applicator onto the inside lip of the tube. Tap tube on a hard surface to let the antifoam settle to the bottom of the tube.

2. **Go to appropriate section for sample type** (see table on preceding page) and follow “Preparation” and “Lysis” steps. After these sample-specific steps, the following protocol takes about 15 min. for the first sample; add 2 min. for each additional sample.

Bind Nucleic Acids to Magnetic Beads

1. Open **MagBead strip tube** by peeling back cover. The first well in the MagBead strip tube has magnetic beads that are black in color.
2. Perform this step for **ALL** protocols **EXCEPT** RNA protocols for Whole Blood, NPS and Throat Swab, NPS and NPW, CSF, and Powder, and DNA/RNA protocol for Sputum (because buffer has already been added to the bead tube):

   Use pipettor to add **800 µL MB binding buffer** to the first well in MagBead strip tube.
3. Perform this step **ONLY for Serum** DNA protocol (to increase concentration of final sample): Use pipettor to remove **100 µL** from the last well (MB elution buffer) in MagBead strip tube.
4. Use a **small transfer pipet** to transfer sample (supernatant) from bead tube to the first well in the MagBead strip tube.
5. Mix sample with beads by pipetting up and down with small transfer pipet.
6. **Binding incubation:** Incubate at room temperature for at least **8 min.** (a longer incubation is acceptable, up to 45 min.).

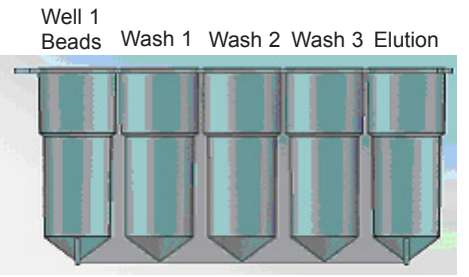
Using the Magnetic Tool

7. Follow the manufacturer’s instructions for the magnetic tool to lock and unlock the magnet once the tip is on the tool.

Wash Magnetic Beads

Note: The same magnetic tip is used for washing and eluting.

Note: Well 1 is on the side of the elongated tab strip tube.



1. To collect the magnetic beads from the first (binding) well, **lock magnet on** and move the Magnetic Tool gently with a circular and up and down motion in the solution for about 30–45 sec.
2. Transfer the magnetic beads into the second well by **unlocking the magnet** and gently moving the Magnetic Tool around in the solution until the magnetic beads come off the magnetic tip into the wash buffer.
3. Transfer more magnetic beads from the first well to the second well by repeating steps 1 and 2.
4. To collect the magnetic beads from the second (wash) well, **lock magnet on** and move the Magnetic Tool with a circular and up and down motion in the solution for about 5–10 sec.
5. Transfer the magnetic beads into the third well and gently release them by **unlocking the magnet** and gently moving the Magnetic Tool around in the solution until the magnetic beads come off the magnetic tip into the wash buffer.
6. To collect the magnetic beads from the third (wash) well, **lock magnet on** and move the Magnetic Tool with a circular and up and down motion in the solution for about 5–10 sec.
7. Transfer the magnetic beads into the fourth well and gently release them by **unlocking the magnet** and gently moving the Magnetic Tool around in the solution until the magnetic beads come off the magnetic tip into the wash buffer.
8. To collect the magnetic beads from the fourth (wash) well, **lock magnet on** and move the Magnetic Tool with a circular and up and down motion in the solution for about 5–10 sec.

Elute Purified Sample

9. Transfer the magnetic beads into the last well and release them by **unlocking the magnet**. Vigorously move the Magnetic Tool around in the elution buffer until all the magnetic beads have come off the tip. Use the end of the tip to break up any magnetic bead clumps in the elution buffer.
10. Incubate magnetic beads in the elution buffer at room temperature for at least **2 min.**
11. To remove magnetic beads, lock magnet on and move the Magnetic Tool with a circular and up and down motion in the solution for about 5–10 sec. Discard beads with tip by ejecting magnetic tool tip.
12. Transfer purified sample to a receiver tube with a pipettor.

Test Purified Sample

13. Test sample as soon as possible. If testing will not be performed immediately, store purified sample at 2–8°C. Storing at 2–8°C longer than 24 hours may reduce sensitivity. For long-term storage, freeze at -20°C. Freezing may reduce sensitivity.

Whole Blood (DNA)

Note: DNA and RNA must be extracted from blood separately. Use of the incorrect protocol may result in incorrect test results.

Acceptable sample: 800 µL whole blood collected with EDTA (ethylenediamine tetraacetic acid) or citrate anticoagulant.

Preparation

1. Use pipettor to add **800 µL whole blood** to medium bead tube containing antifoam.

Lysis and Protease

2. Add **200 µL FLOW protease** to bead tube.
3. Cap tube tightly.
4. Invert bead tube 3 times to mix and let incubate at room temperature for **5 min**.
5. Bead beat for **5 min.** in Disruptor Genie equipped with 2.0 mL tube adaptor, cover in the down position.
6. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Whole Blood (RNA)

Note: DNA and RNA must be extracted from blood separately. Use of the incorrect protocol may result in incorrect test results.

Acceptable sample: 400 µL whole blood collected with EDTA or citrate anticoagulant.

Preparation

1. Use pipettor to add **400 µL whole blood** to bead tube.
2. Use pipettor to add **800 µL MB binding buffer** to bead tube.
3. Use pipettor to add **20 µL VIBE protease** to bead tube.
4. Cap tube tightly.

Lysis

5. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
6. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Serum (DNA)

Acceptable sample: 800 µL serum.

Preparation

1. Use pipettor to add **800 µL serum** to bead tube.
2. Use pipettor to add **80 µL VIBE protease** to bead tube [**NOTE**: If routinely performing serum purifications, it may be necessary to purchase additional VIBE protease].
3. Cap tube tightly and invert three times.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
5. Go to "**Bind Nucleic Acids to Magnetic Beads**" in Main Protocol.

Gastric Wash (DNA)

Acceptable sample: 800 µL gastric wash (gastric lavage)

Preparation

1. Use pipettor to add **800 µL gastric wash** to medium bead tube containing antifoam.

Lysis and Protease

2. Add **200 µL FLOW protease** to bead tube.
3. Cap tube tightly.
4. Invert bead tube 3 times to mix and let incubate at room temperature for **5 min**.
5. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
6. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Culture (DNA/RNA)

Acceptable sample: Plated colonies, plaques, or liquid culture (including blood culture).

Preparation

1. Use pipettor to add **400 µL water** to medium bead tube containing antifoam.
2. **Culture sample**—for plated colonies or plaques: Touch a **Sample Swab** to the colony or plaque and break off the swab at the breakpoint into a bead tube. For culture: Dip a **Sample Swab** in culture media, allow it to soak for 5 sec. and break off the swab at the breakpoint in a bead tube. Alternatively, pipette 40–50 µL liquid culture directly into a bead tube.
3. Cap bead tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
5. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Direct Testing of Bacterial Colonies

Acceptable sample: Plated Colonies

Preparation

1. Use a pipettor to add **500 µL water** to medium bead tube (not containing antifoam).
2. Touch a **Sample Swab** to the colony and break off the swab at the breakpoint into a bead tube.
3. Cap bead tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.

Dilution

5. Add **5 µL bead-beaten material** from bead tube to the last well (elution well) of a **Mag Bead strip tube**.
6. Transfer the full sample from elution well to a small receiver tube with a pipettor.

Test Purified Sample

7. Test sample as soon as possible. If testing will not be performed immediately, store processed sample at 2-8°C. Storing at 2-8°C longer than 24 hours may reduce sensitivity. For long-term storage, freeze at -20°C. Freezing may reduce sensitivity.

Cerebral Spinal Fluid (DNA)

Note: DNA and RNA must be extracted from cerebral spinal fluid (CSF) separately. Use of the incorrect protocol may result in incorrect test results.

Acceptable sample: 800 µL CSF

Preparation

1. Use pipettor to add **800 µL cerebral spinal fluid** to medium bead tube containing antifoam.
2. Cap tube tightly.

Lysis

3. Bead beat for **5 min.** in Disruptor Genie equipped with 2.0 mL tube adaptor, cover in the down position.

4. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Cerebral Spinal Fluid (RNA)

Note: DNA and RNA must be extracted from cerebral spinal fluid (CSF) separately. Use of the incorrect protocol may result in incorrect test results.

Acceptable sample: 400 µL of CSF

Preparation

1. Use pipettor to add **800 µL MB binding buffer** to medium bead tube containing antifoam.
2. Use pipettor to add **400 µL cerebral spinal fluid**.
3. Cap tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.

5. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Sputum (DNA/RNA)

Acceptable sample: 400 µL liquid sputum. Sample a liquid portion of the sputum (may need to cut the pipette tip in order to pipette viscous sputum). A smaller volume of sample can be processed by bringing up the volume to 400 µL with water or PBS, but sensitivity may be reduced.

Preparation

1. Use pipettor to add **800 µL MB binding buffer** to medium bead tube containing antifoam.
2. Use pipettor to add **400 µL sputum** to bead tube.
3. Cap tube tightly.
4. Invert bead tube 3 times and incubate for **15 min.** at room temperature, inverting tube every 5 min.

Lysis and Protease

5. Use pipettor to add **20 µL VIBE protease** to bead tube.
6. Cap tube tightly.
7. Invert bead tube 3 times and incubate for **5 min.** at room temperature.
8. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.

Centrifugation Step

9. Centrifuge bead tube for **5 min.** in a low speed centrifuge **(2000 x *g*).**
10. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Sputum Protocol with Pre-treatment (for DNA only)

To decrease viscosity, sputum samples are pretreated with a 2x concentration of Snap n' Digest before purification.

Acceptable sample: 300 µL of sputum

Preparation

1. Preparation of Snap n' Digest.

   a. Discard approximately 35 mL (1/2 the total volume) of buffer contained in the Snap n' Digest bottle.

   b. With the cap loose, break the ampule inside by squeezing the bottle and mix gently. Once prepared the solution can be used for up to 1 week when stored at room temperature (18-28˚C).
2. Add powdered antifoam to bead tube by scraping a small amount of antifoam from the applicator onto the inside lip of the tube. Tap tube on a hard surface to let the antifoam settle to the bottom of the tube.
3. Transfer 300 µL of sputum from the specimen container and place in the bead tube.

Note: Cutting the end off the pipette tip can aid in pipetting viscous sputum specimens.

4. Add 150 µL of Snap n' Digest, cap tube, and mix by inversion.
5. Incubate at room temperature for approximately 30 min. Mix by inverting or vortexing every 5 to 10 min. The sputum sample should be completely liquefied after this digestion period. If not, digest for another 15 min. with periodic mixing.

Lysis and Protease

6. Use pipettor to add **800 µL** MB binding buffer to the bead tube containing digested sample.
7. Use pipettor to add **20 µL** VIBE protease to the bead tube.
8. Cap tube tightly.
9. Invert bead tube 3 times and incubate for 5 min. at room temperature.

Centrifugation Step

10. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
11. Centrifuge bead tube for **5 min.** in a low speed centrifuge (2000 x g).
12. Go to "**Bind Nucleic Acids to Magnetic Beads**" in Main Protocol in IT 1-2-3™ Platinum Path Sample Purification Kit Instruction Booklet.

Pus Swab and Lymph Node Aspirates (DNA)

Acceptable sample:

- Pus sample on a swab, stored in a dry tube (Copan 168C rayon swab with twisted wire or equivalent is recommended).
- Lymph node aspirate sample (approximately 20 µL).

Preparation

1. Use pipettor to add **400 µL water** to medium bead tube containing antifoam.
2. **Pus swab sample**—Remove swab from its tube. With provided snippers, cut off the head of the swab into a bead tube approximately ¼ in. above the head with snippers. Discard remaining part of the swab. Clean snippers with alcohol wipe.

   Lymph Node Aspirate sample—Use a **Sample Swab** to absorb liquid portion of lymph node aspirate. If no liquid is present, touch sample with the swab 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). Break swab into bead tube at swab breakpoint. If sample is stored on swab other than **Sample Swab**, cut off the head of the swab into a bead tube approximately ¼ in. above the head with provided snippers. Clean snippers with alcohol wipe.
3. Cap bead tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
5. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Nasal Swab (DNA/RNA)

Acceptable sample: Nasal swab on a dry Copan 168C rayon swab and stored in a dry tube. Calcium alginate swabs with aluminum shafts should not be used.

Preparation

1. Use pipettor to add **400 µL water** to medium bead tube containing antifoam.
2. **Nasal swab sample**—Remove swab from its tube. With provided snippers, cut off the head of the swab into a bead tube approximately ¼ in. above the head with snippers. Discard the remaining part of the swab. Clean snippers with alcohol wipe.
3. Cap bead tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
5. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Nasopharyngeal Swab and Nasopharyngeal Wash (RNA)

Acceptable Sample: NPS or NPW sample stored in viral transport media (VTM). (Collection products used for internal studies, or equivalent, are recommended, NPS: Copan 168C rayon swab, NPW: 5-10 mL of 0.9% Sodium Chloride Solution collected in a sterile sample collection container, and Remel Microtest M4-RT VTM.) Note that calcium alginate swabs with aluminum shafts should not be used.

Note: This protocol has been optimized for use with specific JBAIDS freeze-dried reagent kits. Check that the full name of this purification protocol matches the name specified in the JBAIDS freeze-dried reagent product insert.

Preparation

1. Vortex sample in VTM for **5 sec.** using the Disruptor Genie.
2. Use pipettor to add **800 µL** VTM sample to medium bead tube containing antifoam.

Lysis

3. Use pipettor to add **800 µL** MB Binding Buffer to bead tube.
4. Cap tube tightly.
5. Bead beat for **1 min.** in Disruptor Genie equipped with **2.0 mL** tube adaptor, cover in the down position.
6. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Nasopharyngeal Swab and Throat Swab (RNA)

Acceptable Sample: NPS sample or Throat swab sample stored in viral transport media (VTM). (Collection products used for internal studies, or equivalent, are recommended: Copan 168C rayon swab for NPS samples; Hardy Diagnostics HD25806 polyester swab for throat samples; and Remel Microtest M4-RT VTM.) Note: Calcium alginate swabs with aluminum shafts should not be used.

Note: This protocol has been optimized for use with specific JBAIDS freeze-dried reagent kits. Check that the full name of this purification protocol matches the name specified in the JBAIDS freeze-dried reagent product insert.

Preparation

1. Vortex swab in VTM for **5 sec.** using the Disruptor Genie.
2. Use pipettor to add **800 µL** VTM sample to medium bead tube containing antifoam.

Lysis and Protease

3. Use pipettor to add **800 µL** MB Binding Buffer to bead tube.
4. Use pipettor to add **20 µL** VIBE Protease to bead tube.
5. Cap tube tightly.
6. Invert bead tube 3 times and let incubate at room temperature for 5 min.
7. Bead beat for **1 min.** in Disruptor Genie equipped with 2.0 mL tube adaptor, cover in the down position.
8. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Stool (DNA)

Acceptable sample: 1.5 g stool sample. Pretreat as follows:

A. Sample 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 shake or vortex to mix well.

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

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 **400 µL of SCOOP lysis buffer**.
2. Add **400 µL pretreated stool sample** to medium bead tube containing antifoam.

Note: One of the components in SCOOP lysis buffer forms a white precipitate if solution is stored below room temperature (below 18°C). Vigorously rub SCOOP lysis buffer bottle between hands for about 2 min. (or longer) to ensure precipitate gets back into solution.

3. Cap tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.

Protein Precipitation

5. Centrifuge bead tube for **2 min.** in a low speed centrifuge (2000 x *g*).
6. Use pipettor to add **200 µL SCOOP PP1*** to receiver tube.
7. Use a pipettor to transfer liquid supernatant to a clean receiver tube.
8. Mix tube by inverting 10 times.
9. Centrifuge receiver tube for **2 min.**
10. Use pipettor to add **200 µL SCOOP PP2*** to second receiver tube.
11. Use a pipettor to transfer liquid supernatant to second receiver tube.
12. Mix tube by inverting 10 times.
13. Centrifuge sample for **2 min.**
14. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

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Mixed Greens (DNA)

Acceptable sample: 25 g of mixed greens or mixed vegetables washed to yield an 800 µL wash (see Food Wash Protocol below).

Food Wash Protocol

A. Weigh 25 g (+/- 3 g) of mixed greens and transfer to center pocket of a filter blender bag. (If a scale is not available, 25 g of mixed greens is approximately equivalent to ½ cup).

B. Add 100 mL buffer (PBS or other phosphate buffer pH range 7.2–7.4) to the filtered blender bag. This is effectively a five-fold dilution of the sample.

C. Mix and shake bag by hand for 2 min. until greens are well washed with liquid (or stomach for 1 min. set to 200 rpm).

D. Dispose of center filter bag containing solids.

Preparation

1. Use pipettor to add **800 µL liquid wash sample** to medium bead tube containing antifoam.
2. Cap tube tightly.

Lysis

3. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
4. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Milk and Meat (DNA)

Acceptable sample:

- 800 µL milk
- 25 g of meat washed to yield an 800 µL wash (see Food Wash Protocol below)

Note: Protocol was validated for ground beef and tuna salad.

Food Wash Protocol

A. Weigh **25 g (+/- 3 g) of food** and transfer to center pocket of a filter blender bag. (If a scale is not available, 25 g of tuna salad/ground beef is approximately equivalent to ¼ cup).

B. Add **100 mL buffer** (PBS or other phosphate buffer pH range 7.2–7.4) to the filtered blender bag. This is effectively a five-fold dilution of the sample.

C. **For tuna salad:** Mix by gently raising and lowering bag onto a solid surface for **30 sec.** A gentle wash is important as tuna falls apart rapidly.

   For meat: Mix and shake bag by hand for **2 min.** (or stomach for **2 min.** on highest setting).

D. Dispose of center filter bag containing solids.

Preparation

1. Use pipettor to add **800 µL liquid wash sample** to medium bead tube containing antifoam.

Lysis and Protease

2. Add **200 µL FLOW protease** to bead tube.
3. Cap tube tightly.
4. Invert bead tube 3 times to mix and let incubate at room temperature for **5 min.**
5. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
6. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Water (DNA)

Acceptable sample for Water: 800 µL water

Preparation

1. Use pipettor to add **800 µL water sample** to medium bead tube containing antifoam.
2. Cap tube tightly.

Lysis

3. Bead beat for **5 min.** in Disruptor Genie equipped with 2.0 mL tube adaptor, cover in the down position.
4. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Air-into-PBS (DNA/RNA)

Acceptable sample for Air-into-PBS:

800 µL PBS with or without 0.1% Triton® X-100, used to collect air sample or wash dry filter. Devices such as the dry filter unit (DFU) collect air particulates onto a filter. Established protocols are used to transfer the particulates from the filter into PBS. One optimized protocol is described here (wash efficiency of this protocol is not established).

Filter Wash Protocol

A. Place dry filter in a 50 mL conical tube with collection side facing away from the side of the tube.

B. Add **10 mL PBS with 0.1% Triton X-100** to the conical tube.

C. Cap tube and shake by hand for 2 min.

Preparation

1. Use pipettor to add **800 µL liquid sample** to medium bead tube containing antifoam.
2. Cap tube tightly.

Lysis

3. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
4. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Powder (DNA)

Note: DNA and RNA must be extracted from powder separately. Use of the incorrect protocol may result in incorrect test results.

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

Preparation

1. Use pipettor to add **400 µL water** to medium bead tube containing antifoam.
2. Wet a **Sample Swab** with water in bead tube. Touch the powder with only the very tip of the swab. Break swab into bead tube at swab breakpoint.
3. Cap bead tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
5. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Powder (RNA)

Note: DNA and RNA must be extracted from powder separately. Use of the incorrect protocol may result in incorrect test results.

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

Preparation

1. Use pipettor to add **400 µL water** to medium bead tube containing antifoam.
2. Wet a **Sample Swab** with water in bead tube. Touch the powder with only the very tip of the swab. Break swab into bead tube at swab breakpoint.
3. Use pipettor to add **800 µL MB binding buffer** to bead tube.
4. Cap tube tightly.

Lysis

5. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
6. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Surface Swab Protocol (DNA/RNA)

Acceptable sample: Surface swab using a **Sample Swab** or similar swab.

Preparation

1. Use pipettor to add **400 µL water** to medium bead tube containing antifoam.
2. Wet a **Sample Swab** with water in bead tube. Thoroughly wipe a 2-in. x 2-in. surface area to be sampled with swab. Break swab into bead tube at swab breakpoint.

Note: If other samples are taken with a swab other than a **Sample Swab**, cut off the head of the swab into a bead tube approximately ¼ in. above the head with provided snippers. Clean snippers with alcohol wipe.

3. Cap bead tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.
5. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Soil (DNA)

Acceptable sample: 0.5 g of soil.

Preparation

1. Add **0.5 g sample** to medium bead tube containing antifoam (approximately equivalent to filling the medium bead tube to the 1 mL line).
2. Add **800 µL SCOOP lysis buffer**.

Note: One of the components in SCOOP lysis buffer forms a white precipitate if solution is stored below room temperature (below 18°C). Vigorously rub SCOOP lysis buffer bottle between hands for about 2 min. (or longer) to ensure precipitate gets back into solution.

3. Cap tube tightly.

Lysis

4. Bead beat for **5 min.** in Disruptor Genie by placing bead tube in 2.0 mL tube adaptor with cover in the down position.

Protein Precipitation

5. Centrifuge bead tube for **2 min.** in a low speed centrifuge (2000 x *g*).
6. Use pipettor to add **200 µL SCOOP PP1*** to receiver tube.
7. Use a pipettor to transfer liquid supernatant to a clean receiver tube.
8. Mix tube by inverting 10 times.
9. Centrifuge receiver tube for **2 min.**
10. Use pipettor to add **200 µL SCOOP PP2*** to second receiver tube.
11. Use a pipettor to transfer liquid supernatant to second receiver tube.
12. Mix tube by inverting 10 times.
13. Centrifuge sample for **2 min.**

14. Go to **"Bind Nucleic Acids to Magnetic Beads"** in Main Protocol.

Note: Some soil types contain high levels of inhibitors that may not be entirely removed with this protocol. If amplification does not occur, or inhibition controls 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.

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Guidelines

Process	Steps
Processing small volumes	Normalize the volume of sample by adding water or PBS to match the desired volume (based on the acceptable sample volume) of the matrix and organism in question.
Purifying more than one sample at a time	1. Add all samples to the first well of the MagBead strip tubes for binding so all samples complete binding incubation at the same time. 2. Wash second sample while first sample is incubating in elution buffer: a. After step 10 of Main Protocol, eject Magnetic tip into the last well. b. Incubate magnetic beads in the elution buffer at room temperature for at least **2 min.** During elution incubation of previous sample, perform steps 1–10 for the next sample. c. When washing of the second sample is complete, the 2-min. elution incubation should be complete. Using the index finger and thumb, place the Magnetic tip back on the Magnetic Tool by lightly holding at the top of tip and gently sliding the tip back into place. Remove magnetic beads with Magnetic Tool, and discard beads with the tip. d. Continue washing and eluting pairs of samples in this way. ⚠ **WARNING:** Don't mix up tips between samples, as it will lead to contamination.

Process	Steps
Direct testing bead beat only	• **Surface Swab or Powder:** Some surface swab or powder samples do not contain downstream inhibitors and can be tested directly after bead beating. 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 water (for example 40 µL sample plus 360 µL water). In this case, follow the protocol through bead beating and proceed to downstream analysis. Use of inhibition controls is recommended. • **Bacterial Colonies:** Some bacterial colonies can be tested directly after bead beating. Follow protocol through bead beating and immediately after: 1. Add 5 µL bead-beaten sample to a small receiver tube. 2. Add 500 µL water and proceed with downstream analysis. 3. Use of inhibition controls is recommended.
Inhibition Control	Inhibition controls included in a test ensure that inhibitors in a given sample do not inhibit the downstream analysis and that a negative result is accurate. 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**. 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.

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