USER GUIDE

applied biosystems by life technologies™

RapidFinder™ Virus Extraction Kit

Isolation of viral RNA from food and water samples

Optimized for real-time RT-PCR detection of Hepatitis A, Hepatitis E, Norovirus GI, and Norovirus GII

for use with:
BeadRetriever™ System

Catalog Number A25493
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Revision B.0

For testing of Food and Environmental samples only.

life technologies™

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About this guide

Revision history

Revision	Date	Description
B.0	June 2014	Updated and optimized instructions for isolation of RNA from virus particles extracted from food and water samples, using the RapidFinder™ Virus Extraction Kit. Updated document title to reflect new kit name. Updated document template with associated changes to organization and format.
A.0	February 2014	New document. Included instructions for isolation of RNA from virus particles extracted from food and water samples, using off-the-shelf Life Technologies and third-party reagents.

1 Product information

IMPORTANT! Before using the products described in this guide, read and understand the information in the "Safety" appendix in this document.

Product description

The RapidFinder™ Virus Extraction Kit is designed for isolation of high-quality RNA from virus particles that have been extracted and concentrated from food and water samples. The kit uses guanidine thiocyanate disruption of virus particles and automated purification using magnetic silica beads and the BeadRetriever™ System, for optimized recovery of viral RNA that is ready for real-time RT-PCR detection.

Procedure overview

First, food or water samples are collected and processed to extract and concentrate virus particles, according to the sample matrix. For all sample matrices, Mengovirus from the *Mengovirus* Extraction Control Kit[1] is added during processing as a control for virus particle extraction and subsequent RNA isolation steps.

Next, RNA is isolated and purified from the virus particles using the RapidFinder™ Virus Extraction Kit. 500 µL of the processed sample is incubated at 56°C in the presence of Lysis/Binding Solution Concentrate, isopropanol, Carrier RNA, and Bead Mix to disrupt the virus particles and bind the viral RNA to the MyOne™ SILANE Beads. In an automated procedure on the BeadRetriever™ System, the beads (with bound RNA) are washed with Wash Solution 1 and Wash Solution 2, then the RNA is eluted from the beads in 100 µL of Elution Buffer.

The eluted RNA is ready for real-time RT-PCR or storage.

The procedures for extraction and concentration of virus particles have been modified from those described in ISO/TS 15216 (2013), to increase virus recovery and optimize ease of use. The RNA isolation procedure is compliant with ISO/TS 15216, and it can be used downstream of ISO 15216 methods for extraction and concentration of virus particles.

Kit contents and storage

The RapidFinder™ Virus Extraction Kit (Cat. no. A25493) ships in two boxes. Reagents sufficient for 50 samples are supplied.

[1] ceeramTools® kits are developed by Ceeram SAS and distributed by Life Technologies.

Table 1 RapidFinder™ Virus Extraction Kit, Box 1

Component	Volume	Storage
Carrier RNA	300 µL	–25°C to –15°C
Lysis/Binding Enhancer	500 µL	–25°C to –15°C

Table 2 RapidFinder™ Virus Extraction Kit, Box 2

Component	Volume	Storage
Lysis/Binding Solution Concentrate	50 mL	15°C to 30°C
MyOne™ SILANE Beads	500 µL	2°C to 8°C Do not freeze.
Wash Solution 1 Concentrate	33 mL[1]	15°C to 30°C
Wash Solution 2 Concentrate	50 mL[2]	15°C to 30°C[3]
Elution Buffer	5 mL	15°C to 30°C[3]

[1] Final volume; add 11 mL of 100% isopropanol before use.
[2] Final volume; add 40 mL of 100% ethanol before use.
[3] Reported as "Ambient" on the component label.

Required materials not supplied with the kit

Unless otherwise specified, all materials are available from Life Technologies.

MLS: Fisher Scientific or other major laboratory supplier. Visit **www.fisherscientific.com** or contact your local sales representative.

Table 3 Materials required for RNA isolation

Item	Source
BeadRetriever™ System	Cat. no. 15950
BeadRetriever™ Tube Rack	Cat. no. 15952
BeadRetriever™ Tubes and Tips	Cat. no. 15951
DynaMag™-5 Magnet	Cat. no. 12303D
56°C water bath or calibrated incubator	MLS
Benchtop microcentrifuge	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
Adjustable micropipettors, 10 µL to 1000 µL	MLS
Aerosol-resistant pipette tips	MLS
5-mL Eppendorf tubes	Fisher Scientific 14-282-301

Item	Source
Nuclease-free microcentrifuge tubes, 1.5 mL	Cat. no. AM12400, or equivalent
Mengovirus Extraction Control Kit[1]	Cat. no. 4475931
100% isopropanol	MLS
100% ethanol	MLS

[1] ceeramTools® kits are developed by Ceeram SAS and distributed by Life Technologies.

Table 4 Additional materials required for virus particle extraction

Item	Source	Food surfaces	Soft fruits/ vegetables	Herbs/ spices	Water	Bivalve molluscan shellfish
Refrigerated (4±3°C) centrifuge and rotor, capable of: • 15,000 × *g* • Tube volumes ≥20 mL • Handling chloroform-resistant tubes	MLS	—	✓	✓	✓	—
Benchtop centrifuge and rotor, capable of: • 4000 × *g* • Tube volumes of 5 or 15 mL	MLS	—	—	—	—	✓
Benchtop microcentrifuge, 4±3°C, capable of handling chloroform-resistant tubes	MLS	—	✓	✓	✓	✓
Platform rocker, 60±5 rpm, room temperature	MLS	—	✓	✓	—	—
Platform rocker, 60±5 rpm, 4±3°C	MLS	—	✓	✓	✓	—
Shaker, 500 rpm, room temperature	MLS	—	—	—	✓	—
Shaking incubator, 37±1.0°C, 320±20 rpm, or equivalent	MLS	—	—	—	—	✓
Water bath, air incubator, or equivalent, 60±2.0°C	MLS	—	—	—	—	✓
Masterflex peristaltic pump	Sartorius VFP001 (230V) VFP002 (115V)	—	—	—	✓	—

Item	Source	Food surfaces	Soft fruits/ vegetables	Herbs/ spices	Water	Bivalve molluscan shellfish
Vivaflow 50 cassette For use with the Masterflex peristaltic pump	Sartorius VF05P3	—	—	—	✓	—
Aspirator or equivalent apparatus for removing supernatant	MLS	—	✓	✓	✓	✓
Laboratory scale	MLS	—	✓	✓	—	✓
pH meter, for reagent preparation	MLS	—	✓	✓	✓	—
pH testing strips, for pH adjustment of acidic matrices	MLS	—	✓	✓	—	—
Sterile cotton swabs	MLS	✓	—	—	—	—
Sterile scalpels	MLS	—	✓	—	—	—
Equipment for shucking shellfish: • Sterile shucking knife • Heavy duty safety glove • Rubber block	General retail	—	—	—	—	✓
Equipment for dissecting shellfish: • Scissors • Forceps • Razor blades or equivalent equipment for mincing tissue • Sterile Petri dishes	MLS	—	—	—	—	✓
Mesh filter bags, 400 mL	MLS	—	✓	✓	—	—
Pipette filler and pipettes, 5 mL to 25 mL	MLS	—	✓	✓	✓	✓
5-mL Eppendorf tubes[1]	Fisher Scientific 14-282-301	✓	—	—	—	—
Centrifuge tubes, nuclease-free, chloroform-resistant, graduated	MLS	—	50 mL	50 mL	50 mL	5 or 15 mL
(Optional) Chloroform-resistant tubes, 1.5 or 2 mL (if sample is transferred before chloroform:butanol extraction)	MLS	—	✓	✓	✓	✓

Item	Source	Food surfaces	Soft fruits/ vegetables	Herbs/ spices	Water	Bivalve molluscan shellfish
Molecular biology-grade water, for additional reagent preparation[3]	MLS	✓	✓	✓	✓	—
Sterile water (for rehydration of dried samples)	MLS	—	✓	—	—	—
10X PBS, pH 7.2–7.4[2]	Cat. no. 70013032 Cat. no. 70011069	✓	✓	✓	✓	—
Pectinase, 3800 U/mL	Sigma-Aldrich P2611-50 mL, or equivalent	—	✓	✓	—	—
For Proteinase K Solution, 30 U/mg, 0.1 mg/mL[3]	Sigma-Aldrich P-2308, or equivalent	—	—	—	—	✓
HCl, 6 M	MLS	—	✓	✓	—	—
NaOH, 10 M	MLS	—	✓	✓	—	—
For 5X PEG/NaCl[3] • Poly(ethylene glycol) (PEG) 8000 • NaCl	MLS	—	✓	✓	—	—
For Chloroform:Butanol[3] • Chloroform • Butanol	MLS	—	✓	✓	✓	✓
For Glycine Buffer[3] • Glycine • NaCl	MLS	—	—	—	✓	—
For PEG 50%[3]	MLS	—	—	—	✓	—
For TGBE Buffer[3] • Tris base • Glycine • Beef extract powder	MLS	—	✓	✓	—	—

[1] Also required for RNA isolation.
[2] Dilute to 1X concentration before use with molecular biology-grade water.
[3] See Appendix A, "Additional reagent preparation".

2 Extract virus particles from samples

Overview of workflows for virus particle extraction

Reagent volumes, incubation times and temperatures, and centrifugation speeds vary by sample matrix.

Food surfaces	Soft fruit/vegetables	Herbs/spices	Water	Bivalve molluscan shellfish (BMS)
	Add Mengovirus to 25 g of fresh or rehydrated dried samples and treat with pectinase in TGBE Buffer. ▼	Add Mengovirus to 5 g of sample and treat with pectinase inTGBE Buffer. ▼	Add Mengovirus to 1.0–1.5 L of water, and concentrate by filtration through a Vivaflow 50 cassette to ~20 mL. ▼	
	Remove particulates by filtration and centrifugation, then adjust the pH to 7.0. ▼	Remove particulates by filtration and centrifugation, then adjust the pH to 7.0. ▼	Rinse the original container and the Vivaflow 50 cassette with Glycine Buffer. ▼	
Vigorously swab up to 100 cm^2 of the food surface. ▼	Precipitate viral particles with PEG/ NaCl. (Optional overnight stopping point) ▼	Precipitate viral particles with PEG/ NaCl. (Optional overnight stopping point) ▼	Precipitate viral particles with PEG. (Optional overnight stopping point) ▼	Add Mengovirus to 2.0 g of digestive glands and treat with Proteinase K Solution. ▼
Immerse the swab in 1 mL of Lysis/Binding Solution Concentrate, press to release the liquid containing viral particles; repeat 3–4 times with the same aliquot. ▼	Resuspend PEG precipitate in 500 µL of 1X PBS. ▼	Resuspend PEG precipitate in 500 µL of 1X PBS. ▼	Resuspend PEG precipitate in 500 µL of 1X PBS. ▼	Remove particulates by centrifugation, then extract 500 µL of the supernatant with 500 µL of chloroform:butanol. ▼
Add Mengovirus and proceed to RNA isolation.	Extract with 500 µL of chloroform:butanol. ▼ Transfer the aqueous (top) phase to a fresh tube and proceed to RNA isolation.	Extract with 500 µL of chloroform:butanol. ▼ Transfer the aqueous (top) phase to a fresh tube and proceed to RNA isolation.	Extract with 500 µL of chloroform:butanol. ▼ Transfer the aqueous (top) phase to a fresh tube and proceed to RNA isolation.	Transfer the aqueous (top) phase to a fresh tube and proceed to RNA isolation.

Important procedural guidelines for virus particle extraction

- Proceed directly to the RNA isolation procedure after processing samples for virus particle extraction. Do not store processed samples before RNA isolation.
- If available, process at least one sample that is known to be negative for the virus of interest, for use as a negative control sample during downstream RNA isolation (negative process control).
- PEG pellets may be difficult to resuspend by vortexing. If so, pipet up and down using a 1-mL pipette tip with the end cut off.
- To maximize virus recovery for samples requiring chloroform:butanol extraction, perform sample processing and the final chloroform:butanol extraction in the same chloroform-resistant tube.

Collect and process samples: food surfaces

1. Moisten a sterile cotton swab in 1X PBS, and swab the surface of interest (maximum area 100 cm^2), applying pressure while swabbing to detach virus particles.

   Rough surfaces may cause deterioration of the swab, and more than one swab may be required to completely swab the target surface.

2. Record the approximate area swabbed.

3. Immerse each swab in a 5-mL Eppendorf tube containing 1 mL of Lysis/Binding Solution Concentrate, and press the swab against the side of the tube to release liquid.

4. Repeat the immersion/pressing cycle 3 or 4 times in the same aliquot of Lysis/Binding Solution Concentrate, to ensure maximal extraction of virus particles.

   Retain the swab in the sample.

5. Add 10.0±0.1 µL of Mengovirus from the *Mengovirus* Extraction Control Kit, and proceed directly to "Isolate RNA: surface swab samples" on page 18.

Collect and process samples: soft fruit and salad vegetables

1. Using a sterile scalpel, coarsely chop fresh or rehydrated dried fruit and vegetable samples:

Sample type	Sample preparation
Fresh soft fruits or salad vegetables	Coarsely chop 25±0.3 g into pieces approximately 2.5 cm × 2.5 cm × 2.5 cm in size.
Dried or lyophilised samples	1. Rehydrate 5 g of sample in 16 mL of sterile water. 2. Weigh 25±0.3 g of the rehydrated sample for processing. 3. (Optional) If necessary, coarsely chop the rehydrated sample into pieces approximately 2.5 cm × 2.5 cm × 2.5 cm in size.

2. Transfer the sample to the sample compartment of a 400-mL mesh filter bag and add:

Component	Volume per sample
TGBE Buffer, pH 9.5 For acidic matrices (pH <5), use TGBE Buffer, pH 12.0.	40±1 mL
Pectinase, 3800 U/mL	1 mL
Mengovirus, from the *Mengovirus* Extraction Control Kit	10.0±0.1 µL

3. Incubate with constant rocking at approximately 60 rpm for 20±1 minutes at room temperature.

   For acidic matrices, monitor the pH of the filtrate at 10-minute intervals during incubation. If the pH falls below 9.0, adjust to 9.5±0.1 with 10 M NaOH. Extend the total incubation time by 10 minutes for each pH adjustment.

4. Decant the filtrate from the filtered compartment into a centrifuge tube, then centrifuge at 10,000 × *g* for 30±5 minutes at 4±3°C.

   Squeeze the bag while decanting the filtrate, to maximize the liquid obtained.

5. Decant the supernatant into a single clean tube and adjust the pH to 7.0±0.5 with 6 M HCl.

   Use steps of ~200 µL or ~10 drops.

6. Add 0.25 volume of 5X PEG/NaCl solution, mix thoroughly by shaking for 60±5 seconds, then incubate with constant rocking at approximately 60 rpm for 60±5 minutes at 4±3°C.

   STOPPING POINT For convenience, this incubation can be extended overnight.

7. Centrifuge at 10,000 × *g* for 30±5 minutes at 4±3°C.

8. Discard the supernatant, add 500±10 µL of 1X PBS, and vortex to resuspend the pellet.

9. Add 500±10 µL of chloroform:butanol, vortex to mix, and incubate at room temperature for 5±0.5 minutes.

10. Centrifuge at 10,000 × *g* for 15±1 minutes at 4±3°C.

11. Carefully transfer the aqueous phase (top phase) to a fresh tube, and proceed directly to "Isolate RNA: all other sample matrices" on page 19.

Collect and process samples: herbs and spices

1. Transfer 5 g of sample to the sample compartment of a mesh filter bag and add:

Component	Volume per sample
TGBE Buffer, pH 9.5 For acidic matrices (pH <5), use TGBE Buffer, pH 12.0.	50±1 mL
Pectinase, 3800 U/mL	1 mL
Mengovirus, from the *Mengovirus* Extraction Control Kit	10.0±0.1 µL

2. Incubate with constant rocking at approximately 60 rpm for 20±1 minutes at room temperature.

   For acidic matrices, monitor the pH of the filtrate at 10-minute intervals during incubation. If the pH falls below 9.0, adjust to 9.5±0.1 with 10 M NaOH. Extend the total incubation time by 10 minutes for each pH adjustment.

3. Decant the filtrate from the filtered compartment into a centrifuge tube, then centrifuge at 11,000 × *g* for 20±5 minutes at 4±3°C.

4. Decant the supernatant into a single clean tube and adjust the pH to 7.0±0.5 with 6 M HCl.

   Use steps of ~200 µL or ~10 drops.

5. Add 0.25 volume of 5X PEG/NaCl solution, mix thoroughly by shaking for 60±5 seconds, then incubate with constant rocking at approximately 60 rpm for 60±5 minutes at 4±3ºC.

   STOPPING POINT For convenience, this incubation can be extended overnight.

6. Centrifuge at 11,000 × *g* for 30±5 minutes at 4±3°C.

7. Discard the supernatant, add 500±10 µL of 1X PBS, and vortex to resuspend the pellet.

8. Add 500±10 µL of chloroform:butanol, vortex to mix, and incubate at room temperature for 5.0±0.5 minutes.

9. Centrifuge at 13,500 × *g* for 15±1 minutes at 4±3°C.

10. Carefully transfer the aqueous phase (top phase) to a fresh tube, and proceed directly to "Isolate RNA: all other sample matrices" on page 19.

Collect and process samples: water

1. Record the volume of each water sample tested (1.0–1.5 L recommended).

2. Add 10.0±0.1 µL of Mengovirus (from the *Mengovirus* Extraction Control Kit), and shake to mix thoroughly.

3. Following the manufacturer's instructions, filter the sample through a Vivaflow 50 tangential filtration cassette at maximum speed 7, using a peristaltic pump, to achieve a final concentrated volume of 20±5 mL.

4. Transfer the concentrated liquid to a sterile tube and note the volume, V1.

5. Add a volume of Glycine Buffer, V2 = (40 mL–V1), to the original empty container for the water, shake for 5 minutes at approximately 500 rpm, then filter the glycine rinse through the Vivaflow 50 cassette at speed 4 for 15 seconds.

   The rinse step maximizes virus particle recovery.

6. Combine the concentrated sample (total volume V1+V2 = 40 mL) in a sterile tube.

7. Add 10 mL of PEG 50% Solution, mix thoroughly by shaking for 60±5 seconds, then incubate with constant rocking at approximately 60 rpm for 60±5 minutes at 4±3°C.

   STOPPING POINT For convenience, this incubation can be extended overnight.

8. Centrifuge at 11,000 × *g* for 30±5 minutes at 4±3°C.

9. Discard the supernatant, add 500±10 µL of 1X PBS, and vortex to resuspend the pellet.

10. Add 500±10 µL of chloroform:butanol, vortex to mix, and incubate at room temperature for 5±0.5 minutes.

11. Centrifuge at 13,500 × *g* for 15±1 minutes at 4±3°C.

12. Carefully transfer the aqueous phase (top phase) to a fresh tube, and proceed directly to "Isolate RNA: all other sample matrices" on page 19.

Collect and process samples: bivalve molluscan shellfish (BMS)

Use live (recommended) or frozen, undamaged, bivalve molluscan shellfish (BMS).

CAUTION! When opening the shells, protect the hand holding the BMS with a heavy-duty safety glove.

1. Remove mud adhering to the shell, but do not re-immerse BMS in water.

2. Open the shells of at least 10 BMS with a sterile shucking knife.

3. Dissect out the digestive glands from all BMS using scissors and forceps to obtain a 2.0±0.2-g portion, and transfer the tissue to a clean petri dish.

   We recommend preparing a second tissue sample for retesting, if necessary. Store the second tissue sample at or below –20°C. For long-term storage, we recommend –80°C.

4. Finely chop the digestive glands with a razor blade or equivalent equipment to a paste-like consistency, and transfer a 2.0±0.2-g portion to a 5- or 15-mL centrifuge tube.

5. Add the following components to the sample and mix.

Component	Volume per sample
Mengovirus, from the *Mengovirus* Extraction Control Kit	10.0±0.1 µL
Proteinase K Solution, 30 U/mg, 0.1 mg/mL	2.0±0.2 mL

6. Incubate in a shaking incubator at approximately 320 rpm for 60±5 minutes at 37±1.0°C.

7. Transfer to a 60±2.0°C water bath or equivalent, and incubate for 15±1 minutes.

8. Centrifuge at 3000 × *g* for 5±0.5 minutes, decant the supernatant into a clean tube, then measure and record the volume of supernatant in mL.

   The volume is used in determining the recovery of Mengovirus.

9. Add 500±10 µL of chloroform:butanol to 500±10 µL of supernatant, vortex to mix, and incubate at room temperature for 5±0.5 minutes.

10. Centrifuge at 13,500 × *g* for 15±1 minutes at 4±3°C.

11. Carefully transfer the aqueous phase (top phase) to a fresh tube, and proceed directly to "Isolate RNA: all other sample matrices" on page 19.

3 Isolate RNA from virus particles

Overview of workflows for RNA isolation

Food surface samples	All other sample matrices
Combine sample (contains Lysis/ Binding Solution Concentrate) and Carrier RNA.	Combine sample, Lysis/Binding Solution Concentrate, isopropanol, Carrier RNA, and Bead Mix.
▼	▼
Incubate for 30±5 min at 56°C.	Incubate for 30±5 min at 56°C.
▼	▼
Prepare the BeadRetriever™ Tube Rack with Wash Solution 1, Wash Solution 2, and Elution Buffer.	Prepare the BeadRetriever™ Tube Rack with Wash Solution 1, Wash Solution 2, and Elution Buffer.
▼	
Add isopropanol and Bead Mix, and incubate for 10 min at room temp.	▼
▼	

Capture the RNA-bound beads on a DynaMag™-5 Magnet.

▼

Resuspend the beads in Wash Solution 1 and transfer to Well 1 of the BeadRetriever™ Tube Rack.

▼

Load and run the BeadRetriever™ instrument.

▼

Transfer the final supernatant to a clean tube.

Important procedural guidelines for RNA isolation

- Isolate RNA from a sample that is known to be negative for the virus of interest (negative process control sample), at a frequency determined by the laboratory's quality assurance procedures.

- If a negative process control sample is not available, prepare at least one mock-purified sample for use as a negative extraction control: use 500±10 µL of Nuclease-free Water in place of the unknown sample in the RNA isolation procedure.

Before first use of the kit

Prepare Wash Solutions

Prepare Wash Solutions from the concentrates, then check the box on the labels to indicate that the Wash Solution has been completed.

- Wash Solution 1: Add 11 mL of 100% isopropanol to the bottle of Wash Solution 1 Concentrate and mix thoroughly by shaking.
- Wash Solution 2: Add 40 mL of 100% ethanol to the bottle of Wash Solution 2 Concentrate and mix thoroughly by shaking.

Store the completed Wash Solutions at room temperature.

Obtain the BeadRetriever™ script

Contact your local Thermo Fisher Scientific representative to obtain the processing script MMRNA_DNA_HV_Wash_V1 for the BeadRetriever™ instrument.

Before each use of the kit: prepare Bead Mix

Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MyOne™ SILANE Beads	10 µL
Lysis/Binding Enhancer	10 µL

Isolate RNA: surface swab samples

1. Add 6 µL of Carrier RNA to the swab sample, and incubate for 30±5 minutes at 56°C.

   To maximize viral RNA recovery, the swab remains in the sample through lysis at 56°C. To avoid adsorption of the beads to swab fibers, isopropanol and Bead Mix are added after the swab is removed in step 3.

2. While the samples are incubating at 56°C, prepare the BeadRetriever™ Tube Rack for the appropriate number of samples:

Well	Component	Volume
Well 2	Wash Solution 1	300 µL
Well 3	Wash Solution 2	450 µL
Well 4	Wash Solution 2	450 µL
Well 5	Elution Buffer	100 µL

3. When the 56°C incubation is complete, remove the swab from the sample, add the following components, vortex, and incubate for 10 minutes at room temperature.

Component	Volume
100% isopropanol	1 mL
Bead Mix	20 µL

4. Vortex the sample, place it on a DynaMag™-5 Magnet until the solution clears (about 3 minutes), and remove the supernatant.

5. Resuspend the bead pellet in 300 µL of Wash Solution 1 and transfer the resuspended beads to Well 1 of the prepared BeadRetriever™ Tube Rack.

6. Place the BeadRetriever™ Tube Rack in the BeadRetriever™ instrument, select script **MMRNA_DNA_HV_Wash_V1**, and start the run.

7. When the run is complete, transfer the supernatant (~100 µL), containing the RNA, to a clean tube.

Isolate RNA: all other sample matrices

1. Combine the following components in a 5-mL Eppendorf tube and vortex to mix.

Component	Volume
Sample	500 µL
Lysis/Binding Solution Concentrate	1 mL
100% isopropanol	1 mL
Carrier RNA	6 µL
Bead Mix	20 µL

2. Incubate for 30±5 minutes at 56°C.

3. While the samples are incubating at 56°C, prepare the BeadRetriever™ Tube Rack for the appropriate number of samples:

Well	Component	Volume
Well 2	Wash Solution 1	300 µL
Well 3	Wash Solution 2	450 µL
Well 4	Wash Solution 2	450 µL
Well 5	Elution Buffer	100 µL

4. When the 56°C incubation is complete, vortex the sample, place it on a DynaMag™-5 Magnet until the solution clears (about 3 minutes), and remove the supernatant.

5. Resuspend the bead pellet in 300 µL of Wash Solution 1 and transfer the resuspended beads to Well 1 of the prepared BeadRetriever™ Tube Rack.

6. Place the BeadRetriever™ Tube Rack in the BeadRetriever™ instrument, select script **MMRNA_DNA_HV_Wash_V1**, and start the run.

7. When the run is complete, transfer the supernatant (~100 µL), containing the RNA, to a clean tube.

Real-time RT-PCR detection and storage of the RNA

The RNA is ready for real-time RT-PCR using one of the following kits:

- Hepatitis A Virus Detection Kit[2] (Cat. no. 4475930)
- Hepatitis E Virus Detection Kit[2] (Cat. no. 4489312)
- Norovirus GI Detection Kit[2] (Cat. no. 4475928)
- Norovirus GII Detection Kit[2] (Cat. no. 4475929)

Alternatively, store the RNA:

- At 5±3°C for up to 8 hours.
- Below –15°C for up to 6 months.
- At –80±5°C for longer-term storage.

[2] ceeramTools® kits are developed by Ceeram SAS and distributed by Life Technologies.

A Additional reagent preparation

Unless otherwise specified, prepared reagents are stable for 6 months.

5X PEG/NaCl

1. Dissolve the following in 450±5 mL of molecular biology grade water, heating gently if necessary.

Component	Amount
Poly(ethylene glycol) (PEG) 8000	500±2 g
NaCl	87±1 g

2. Adjust the volume to 1000±10 mL with molecular biology grade water and mix well.
3. Autoclave to sterilize.

Chloroform:butanol (1:1)

Combine the following reagents and mix thoroughly.

Component	Amount
Chloroform	10.0±0.1 mL
Butanol	10.0±0.1 mL

Glycine Buffer, pH 9.5

1. Dissolve the solids in the molecular biology grade water.

Component	Amount
NaCl	9.0±0.5 g
Glycine	3.75±0.5 g
Molecular biology grade water	1000±1 mL

2. Adjust the pH to 9.5±0.2 at 25°C.
3. Autoclave to sterilize.

PEG 50% Solution [50% (w/v) PEG 8000]

1. Dissolve the solids in 450±5 mL of molecular biology grade water, heating gently if necessary.

Component	Amount
Poly(ethylene glycol) (PEG) 8000	500±2 g
Molecular biology grade water	as required

2. Adjust the volume to 1000±10 mL with molecular biology grade water and mix thoroughly.
3. Autoclave to sterilize.

Proteinase K Solution, 30 U/mg, 0.1 mg/mL

It is important to use Proteinase K with a specific activity of 30 U/mg, for maximum virus recovery.

Dissolve the Proteinase K in the molecular biology grade water, and mix thoroughly.

Component	Amount
Proteinase K, 30 U/mg	20.0±0.1 mg
Molecular biology grade water	200±2 mL

Store Proteinase K Solution in working volumes at –20±5°C for up to 6 months.

Once defrosted, store at 4±2°C and use within 1 week.

Tris Glycine 1% Beef Extract (TGBE) Buffer

1. Dissolve the solids in the water.

Component	Amount
Tris base	12.1±0.2 g
Glycine	3.8±0.1 g
Beef extract	10.0±0.1 g
Molecular biology grade water	1000±1 mL

2. Adjust the pH to 9.5±0.2 or 12.0±0.2 at 25°C.
   - Acidic soft fruit and salad vegetable matrices (pH <5) require TGBE Buffer, pH 12.0.
   - Herbs and spices, and other soft fruit and salad vegetable matrices require TGBE Buffer, pH 9.5.
3. Autoclave to sterilize.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Food Safety support

Website: **www.lifetechnologies.com/foodsafety**

Support email: **foodsafety@lifetech.com**

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit **www.lifetechnologies.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

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References

ISO. 2013. Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 1: Method for quantification. Reference number 15216-1.

Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 2: Method for qualitative detection. Reference number 15216-2.