OMNIgene™·XTRACT ULTRA

Extraction of microbial DNA and RNA from vaginal, skin, fecal and saliva samples

REF **OM-XTU-50**

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Purpose

The OMNIgene™•XTRACT ULTRA extraction kit allows for the extraction of DNA and RNA from microbiome samples collected and stabilized in OMNIgene™•ORAL (OMR-110, OMR-120, OMR-610), OMNIgene™•VAGINAL (OMR-130), OMNIgene™•SKIN (OMR-140) and OMNIgene™•GUT DNA and RNA (OMR-205) devices, as well as unstabilized skin, vaginal and oral microbiome samples.

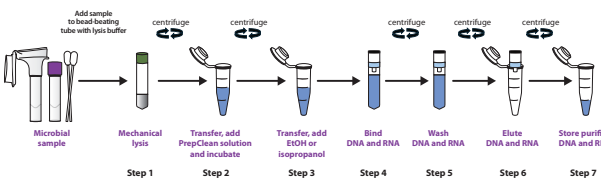
The OMNIgene™•XTRACT ULTRA kit is intended for molecular biology applications and is for research use only. This kit is not intended for diagnostic applications.

Materials

Store all kits components and reagents at room temperature (15°C-30°C/59°F–86°F).

Component	Quantity
Lysis buffer	30 mL
PrepClean solution	19 mL
Bead-beating tubes	50 units (1 bag)
Silica columns	50 units (1 bag)
1.5 mL tubes	100 units (2 bags of 50 units)
Elution tubes	50 units (1 bag)

Workflow overview



Protocol A: Extraction of DNA and RNA from oral, vaginal and skin microbiome samples

Supported sample types	
DNA Genotek™ collection device(s)	OMNIgene™•ORAL (OMR-110, OMR-120, OMR-610)
	OMNIgene™•VAGINAL (OMR-130)
	OMNIgene™•SKIN (OMR-140)
Other sample types	Frozen swabs (without buffer)
	Frozen swabs in phosphate buffered saline (PBS), saline or viral transport media (VTM)

Equipment and reagents to be supplied by user

- Water bath or air incubator set to 50°C
- Pipettors (1 µL-10 µL, 20 µL-200 µL, 100 µL-1,000 µL)
- Microcentrifuge (up to 14,000 x *g*)
- Vortex and vortex adapter for bead beating, compatible with microcentrifuge tubes
- Proteinase K (PK) enzyme solution (80 mg/mL)
- 99% isopropanol
- 70% ethanol
- Nuclease-free water

Notes before starting

- The lysis buffer is provided as a 2x concentrate. When using the kit for the first time, add 30 mL of nuclease-free water to the lysis buffer and mix by inversion.
- Prepare PK stock solution of 80 mg/mL by dissolving lyophilized PK (specific activity > 30 mAU/mg) in nuclease-free water. Store in aliquots at -20°C.

Pre-processing for DNA Genotek™ OMR-610 devices

1. Incubate the entire sample in the original tube at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.

   Notes:

   • Incubation may be performed any time between sample receipt and extraction.

   • This step does not need to be repeated for extraction of subsequent aliquots.

2. Proceed to Protocol A, extraction procedure step 1.
   Note: Samples can be stored at room temperature or frozen at -20°C until ready to extract.

Pre-processing for DNA Genotek™ OMR-110, OMR-120, OMR-130 and OMR-140 devices

1. Add 5 µL of PK solution (80 mg/mL) to the OMNIgene™ collection tube and vortex.
2. Incubate the entire sample in the original tube at 50°C for 1 hour in a water bath or for 2 hours in an air incubator. Ensure that the swab tip is in contact with the stabilization solution during this step.
   Notes:
   • Incubation may be performed any time between sample receipt and extraction.
   • This step does not need to be repeated for extraction of subsequent aliquots.
3. Squeeze out as much of the liquid as possible by pressing the swab on the inner wall of the collection tube and discard the swab.
4. Cap the collection tube and proceed to Protocol A, extraction procedure step 1.
   Note: PK-treated samples can be stored at room temperature or frozen at -20°C until ready to extract.

Pre-processing for non-DNA Genotek™ devices

Swabs (without buffer)

1. Cut swab using sterile scissors. Transfer cut swab to a 1.5 mL microcentrifuge tube (not provided).
2. Add 1,000 µL lysis buffer and 5 µL of PK solution (80 mg/mL) to the tube and vortex.
3. Incubate the entire sample at 50°C for 1 hour in a water bath or for 2 hours in an air incubator. Ensure that the swab tip is in contact with the stabilization solution during this step.
4. Squeeze out as much of the liquid as possible by pressing the swab on the inner wall of the tube. Discard swab and cap the tube.
5. Proceed immediately to Protocol A, extraction procedure step 1.

Frozen swabs (in PBS, saline or viral transport media)

1. Add 1 volume of lysis buffer and 5 µL of PK solution (80 mg/mL) to the tube containing the swab and vortex.
   Note: To ensure there is enough remaining lysis buffer volume to support 50 extractions, use a maximum of 1 mL per sample for this step.
2. Incubate the entire sample at 50°C for 1 hour in a water bath or for 2 hours in an air incubator. Ensure that the swab tip is in contact with the solution during this step.
3. Squeeze out as much of the liquid as possible by pressing the swab on the inner wall of the tube. Discard swab and cap the tube.
4. Proceed immediately to Protocol A, extraction procedure step 1.

Extraction procedure

1. Vortex sample tube at maximum speed for 10 seconds and transfer a 250 µL-1,000 µL aliquot[1] of the sample to be extracted to a bead-beating tube.
2. Bring final liquid volume to 1,000 µL using lysis buffer. (e.g., If processing a 500 µL aliquot of a sample, add 500 µL of lysis buffer.)
3. Cap the bead-beating tube and secure it to the vortex adapter, with the cap oriented toward the center. Vortex for 15 minutes at maximum speed.
4. Centrifuge the bead-beating tube for 2 minutes at 14,000 x *g*. Transfer the entire volume of supernatant to a 1.5 mL tube without disturbing the pellet.
5. Add 160 µL PrepClean solution and vortex for 10 seconds at maximum speed.
6. Incubate tube for 5 minutes at room temperature and centrifuge for 2 minutes at 14,000 x *g*.
7. Transfer the entire volume of supernatant to a new 1.5 mL tube without disturbing the pellet.
8. Add 400 µL isopropanol and vortex briefly to mix.
9. Load 750 µL lysate onto a silica column and centrifuge for 1 minute at 14,000 x *g*. Discard flow-through and place the silica column back into the 2 mL collection tube.
   Note: Do not discard the 2 mL collection tube as it will be re-used for steps 10-12.

1 For oral (OMR-120, OMR-610) and vaginal (OMR-130) samples, the recommended input is 250 µL-500 µL. For skin (OMR-140) samples, the recommended input is 1,000 µL. Input volume can be adjusted depending on expected yield and downstream assay requirements.

10. Repeat step 9 to process remaining lysate.
11. Add 750 µL 70% ethanol to the silica column and centrifuge for 1 minute at 14,000 x *g*. Discard flow-through.
12. To fully dry the silica column, centrifuge for 1 minute at 14,000 x *g*.
13. Place the silica column in an elution tube.
14. Add 100 µL nuclease-free water to the center of the silica column membrane and incubate for 1 minute at room temperature.
    Note: To maximize DNA concentrations for lower biomass samples, such as skin, sample can be eluted in 50 µL nuclease-free water.
15. Centrifuge for 1 minute at 14,000 x *g*. Discard the silica column and store co-eluted DNA and RNA at -80°C.

Protocol B: Extraction of DNA and RNA from OMNIgene™•GUT DNA and RNA (OMR-205) devices

Supported sample types	
DNA Genotek™ collection device(s)	OMNIgene™•GUT DNA and RNA (OMR-205)

If you are interested in extracting DNA and RNA from unstabilized fecal samples, contact your Account Manager or email info@dnagenotek.com.

Equipment and reagents to be supplied by user

- Pipettors (20 µL-200 µL, 100 µL-1,000 µL)
- Microcentrifuge (up to 14,000 x *g*)
- Vortex and vortex adapter for bead beating, compatible with microcentrifuge tubes
- 95%-100% ethanol
- 70% ethanol
- Nuclease-free water

Extraction procedure

1. Vortex an OMNIgene™•GUT DNA and RNA collection tube at maximum speed for 10 seconds.
2. Transfer a 250 µL aliquot of sample from the OMNIgene™•GUT DNA and RNA tube to a bead-beating tube and add 1,000 µL nuclease-free water.
3. Cap the bead-beating tube and secure it to the vortex adapter, with the cap oriented toward the center. Vortex for 15 minutes at maximum speed.
4. Centrifuge the bead-beating tube for 2 minutes at 14,000 x *g*. Transfer 900 µL supernatant to a 1.5 mL tube without disturbing the pellet.
5. Add 180 µL PrepClean solution and vortex for 10 seconds at maximum speed.
6. Incubate tube for 5 minutes on ice and centrifuge for 2 minutes at 14,000 x *g*.
7. Transfer up to 900 µL supernatant to a new 1.5 mL tube without disturbing the pellet.
8. Add 450 µL 95% ethanol and vortex briefly to mix.
9. Load 750 µL lysate onto a silica column and centrifuge for 1 minute at 14,000 x *g*. Discard flow-through and place the silica column back into the 2 mL collection tube.
   Note: Do not discard the 2 mL collection tube as it will be re-used for steps 10-12.

10. Repeat step 9 to process the remaining lysate through the column.
11. Add 750 µL 70% ethanol to the silica column and centrifuge for 1 minute at 14,000 x *g*. Discard flow-through.
12. To fully dry the silica column, centrifuge for 1 minute at 14,000 x *g*.
13. Place the silica column in an elution tube.
14. Add 100 µL nuclease-free water to the center of the silica column membrane and incubate for 1 minute at room temperature.
15. Centrifuge for 1 minute at 14,000 x *g*. Discard the silica column and store co-eluted DNA and RNA at -80°C.

Post-extraction processing

The OMNIgene™•XTRACT ULTRA kit extracts DNA and RNA in a single fraction; therefore, the eluate must undergo additional processing steps for applications that require pure DNA or RNA.

For DNA

1. Following manufacturer's specifications, treat an aliquot of the eluate with RNase A.
2. Quantify DNA using a fluorescence-based assay, such as Quant-iT PicoGreen dsDNA Reagent (Thermo Fisher Scientific, Cat. No. P7589). **Note:** Quantification of DNA using absorbance-based methods can result in an inaccurate measurement.

For RNA

1. Following manufacturer's specifications, perform a DNase treatment and purify the RNA using a silica column-based clean-up kit.
2. Quantify RNA using absorbance or a fluorescence-based assay, such as Quant-iT RiboGreen RNA Reagent (Thermo Fisher Scientific, Cat. No: R32700).

Troubleshooting

Problem		Solution
Low DNA/ RNA yields	**Sample-related**	1. Ensure that the sample was collected properly. Poor sample collection can negatively impact yields. For sample collection with DNA Genotek™ OMNIgene™•GUT (OMR-205, OMR-110, OMR-120, OMR-130, OMR-140 and OMR-610) devices, follow the instructions provided. 2. Prior to extraction, ensure PK incubation was performed. PK treatment helps release the material trapped in the swab to improve yields. 3. Ensure that the sample was properly stored post-collection. Unstabilized samples are prone to degradation and may result in low yields. 4. Yields vary widely across sample types, sites and donors. Some samples will naturally yield lower amounts of nucleic acids than others. Check if yields match what is typically expected for the sample type.
	Extraction-related	1. Ensure that an appropriate amount of sample was used as input into the extraction.[1] 2. Ensure the step to dry the silica column was performed to remove residual ethanol prior to eluting nucleic acids. 3. When working with lower biomass samples (i.e., skin samples), lower the elution volume to increase DNA and RNA concentrations.

1 For oral (OMR-120, OMR-610) and vaginal (OMR-130) samples, the recommended input is 250 µL-500 µL. For skin (OMR-140) samples, the recommended input is 1,000 µL. Input volume can be adjusted depending on expected yield and downstream assay requirements.

Troubleshooting

Problem		Solution
DNA and/or RNA appear degraded	**Sample-related**	1. Ensure that the sample was collected properly. Poor sample collection can negatively impact nucleic acid quality. For sample collection with DNA Genotek™ OMNIgene™•GUT (OMR-205, OMR-110, OMR-120, OMR-130, OMR-140 and OMR-610) devices, follow the instructions provided. 2. Ensure that the sample was properly stored post-collection. Samples stored without stabilization solution are prone to degradation.
	Extraction-related	1. Ensure that the recommended bead-beating conditions were used for lysis. Excessive bead beating can lead to shearing of nucleic acids. 2. Contaminated reagents can introduce nucleases in samples. Use a clean aliquot of nuclease-free water for elution.

Troubleshooting

Problem		Solution
Low purity/ presence of inhibitor	**Sample-related**	1. Ensure that the sample was collected properly. Collecting an excess amount of sample can lead to carryover of inhibitors in the extracted material.
	Extraction-related	1. When working with samples suspected of having higher amounts of inhibitors, perform PrepClean incubation on ice for a longer period of time (e.g., 10 minutes). 2. Do not disturb the pellet/beads when transferring the lysate after bead beating and PrepClean treatment. If large amounts of floating debris are observed after the first centrifugation, before proceeding to the next step, invert the tube several times and centrifuge for an additional 2 minutes at 14,000 x *g*.

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Some DNA Genotek products may not be available in all geographic regions.

All DNA Genotek protocols, white papers and application notes are available in the support section of our website at www.dnagenotek.com.

Label legend:

REF	Catalog number
	Manufacturer
	Consult package insert
LOT	Lot number
	Use by
15°C – 30°C 59°F – 86°F	Storage instructions

Patent (www.dnagenotek.com/legalnotices)

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PD-HB-50 v1/2025-05